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 NEWS  3  Apr 09  BEILSTEIN: Reload and Implementation of a New Subject Area
 NEWS  4  Apr 09  ZDB will be removed from STN
 NEWS  5  Apr 19  US Patent Applications available in IFICDB, IFIPAT, and
IFIUDB
 NEWS  6  Apr 22  Records from IP.com available in CAPLUS, HCAPLUS, and
ZCAPLUS
 NEWS  7  Apr 22  BIOSIS Gene Names now available in TOXCENTER
 NEWS  8  Apr 22  Federal Research in Progress (FEDRIP) now available
 NEWS  9  Jun 03  New e-mail delivery for search results now available
 NEWS 10  Jun 10  MEDLINE Reload
 NEWS 11  Jun 10  PCTFULL has been reloaded
 NEWS 12  Jul 02  FOREGE no longer contains STANDARDS file segment
 NEWS 13  Jul 22  USAN to be reloaded July 28, 2002;
                  saved answer sets no longer valid
 NEWS 14  Jul 29  Enhanced polymer searching in REGISTRY
 NEWS 15  Jul 30  NETFIRST to be removed from STN
 NEWS 16  Aug 08  CANCERLIT reload
 NEWS 17  Aug 08  PHARMAMarketLetter(PHARMAML) - new on STN
 NEWS 18  Aug 08  NTIS has been reloaded and enhanced
 NEWS 19  Aug 19  Aquatic Toxicity Information Retrieval (AQUIRE)
                  now available on STN
 NEWS 20  Aug 19  IFIPAT, IFICDB, and IFIUDB have been reloaded
 NEWS 21  Aug 19  The MEDLINE file segment of TOXCENTER has been reloaded
 NEWS 22  Aug 26  Sequence searching in REGISTRY enhanced
 NEWS 23  Sep 03  JAPIO has been reloaded and enhanced
 NEWS 24  Sep 16  Experimental properties added to the REGISTRY file
 NEWS 25  Sep 16  Indexing added to some pre-1967 records in CA/CAPLUS
 NEWS 26  Sep 16  CA Section Thesaurus available in CAPLUS and CA
 NEWS 27  Oct 01  CASREACT Enriched with Reactions from 1907 to 1985
 NEWS 28  Oct 21  EVENTLINE has been reloaded
 NEWS 29  Oct 24  BEILSTEIN adds new search fields
 NEWS 30  Oct 24  Nutraceuticals International (NUTRACEUT) now available on
STN
 NEWS 31  Oct 25  MEDLINE SDI run of October 8, 2002

 NEWS EXPRESS  October 14 CURRENT WINDOWS VERSION IS V6.01,
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               AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
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=> nucleocapsid
L1 8446 NUCLEOCAPSID

=> pseudo and L1
L2 12 PSEUDO AND L1

=> eukaryotic (w) virus
L3 148 EUKARYOTIC (W) VIRUS

=> L3 and L2
L4 0 L3 AND L2

=> flavivirus and L2
L5 0 FLAVIVIRUS AND L2

=> "hepatitis C virus"
L6 30819 "HEPATITIS C VIRUS"

=> L6 and L2
L7 2 L6 AND L2

=> virus and L2
L8 12 VIRUS AND L2

=> tRNA and L2
L9 0 TRNA AND L2

=> tRNA
L10 42320 TRNA

=> L1 and L10
L11 159 L1 AND L10

=> L11 and L2
L12 0 L11 AND L2

```
=> L6 and L11
L13          1 L6 AND L11

=> "large spheral viral like particle"
L14          0 "LARGE SPHERAL VIRAL LIKE PARTICLE"

=> "viral like particel"
L15          0 "VIRAL LIKE PARTICEL"

=> "viral like particle"
L16         54 "VIRAL LIKE PARTICLE"

=> L16 and pseudo
L17          1 L16 AND PSEUDO

=> D L17 IBIB TI SO AU ABS 1

L17  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         1968:473932  CAPLUS
DOCUMENT NUMBER:          69:73932
TITLE:                    The self-assembly of a nucleic-acid free
                          pseudo-top component for a small spherical
                          virus
AUTHOR(S):                Bancroft, J. B.; Wagner, G. W.; Bracker, C. E.
CORPORATE SOURCE:         Purdue Univ., Lafayette, Indiana, USA
SOURCE:                   Virology (1968), 36(1), 146-9
                          CODEN: VIRLAX
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
TI   The self-assembly of a nucleic-acid free pseudo-top component
     for a small spherical virus
SO   Virology (1968), 36(1), 146-9
     CODEN: VIRLAX
AU   Bancroft, J. B.; Wagner, G. W.; Bracker, C. E.
AB   Protein, isolated from cowpea chlorotic mottle virus, when dialyzed
     overnight at 6.degree. against 0.2M NaCl-0.01M NaOAc, pH 5.0, formed
     nucleic acid-free spheres, which unlike any naturally occuring plant
virus
     top component, differed in charge from the virus.  The particles were
52S,
     had a dry diam. of 250 A., and an electrophoretic mobility in 0.2M NaCl
of
     4.3 .times. 10-5 cm.2v.-1sec.-1.  The particles did not differ
     antigenically from the virus.


=> L16 and L11
L18          0 L16 AND L11

=> D L13 IBIB TI SO AU ABS 1

L13  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2002:357237  CAPLUS
DOCUMENT NUMBER:          137:89946
TITLE:                    Conformational Changes Accompanying Self-Assembly of
                          the Hepatitis C Virus
                          Core Protein
AUTHOR(S):                Kunkel, Meghan; Watowich, Stanley J.   Inventor
CORPORATE SOURCE:         Department of Human Biological Chemistry & Genetics
                          and the Sealy Center for Strucutral Biology,
```

University of Texas Medical Branch, Galveston, TX, 77555, USA
SOURCE: Virology (2002), 294(2), 239-245
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Conformational Changes Accompanying Self-Assembly of the **Hepatitis C Virus** Core Protein
SO Virology (2002), 294(2), 239-245
CODEN: VIRLAX; ISSN: 0042-6822
AU Kunkel, Meghan; Watowich, Stanley J.
AB Although a no. of recent studies have suggested that the function of the **hepatitis C virus** (HCV) core protein may be both to package the viral genome and to modulate host cellular processes, little is known of the structure of the core protein necessary to accomplish these functions. Using in vitro assembled particles that mimic essential features of native HCV **nucleocapsids**, we report the earliest structural information of the HCV core protein and its **nucleocapsid**. The core protein is proteinase-resistant when assembled into **nucleocapsid**-like particles or complexed with nucleic acid in vitro. In contrast, the highly basic amino terminus of the free core protein is sensitive to proteolytic digestion. The hydrophobic carboxyl-terminal region of the core protein stabilizes the structure of the free core protein but is not required to stabilize core protein assembled into **nucleocapsid**-like particles or complexed to nucleic acid. Significantly, the carboxyl-terminal region is sufficient, but not necessary, to fold the core protein into a stable structure. These data are consistent with a model of a partially flexible HCV core protein that undergoes extensive conformational changes upon binding to nucleic acid and assembling into **nucleocapsid** particles. In addn., the susceptibility of **nucleocapsid** particles to RNase digestion suggests that RNA-core interactions may stabilize HCV **nucleocapsids**.
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> D L7 IBIB TI SO AU ABS 1-2

L7 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:56543 CAPLUS
DOCUMENT NUMBER: 126:101682
TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of **hepatitis C virus**
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, 00040, Italy
SOURCE: Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Chimeric Sindbis viruses dependent on the NS3 protease of

hepatitis C virus
SO Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
AB The **hepatitis C virus** (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for anal. of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temp. dependence in the formation of lysis plaques yet stably expressed NS3.

L7 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:81269 BIOSIS
DOCUMENT NUMBER: PREV199799387972
TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of **hepatitis C virus**.
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P. Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy
SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Chimeric Sindbis viruses dependent on the NS3 protease of **hepatitis C virus**.
SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.
ISSN: 0022-538X.
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
AB The **hepatitis C virus** (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for analysis of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences

coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

=> D L2 IBIB TI SO AU ABS 1-12

L2 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:652537 CAPLUS
TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**
AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel
CORPORATE SOURCE: Department of Genetics and Microbiology, University of Geneva School of Medicine, Centre Medicale Universitaire, Geneva, CH1211, Switz.
SOURCE: RNA (2002), 8(8), 1056-1067
CODEN: RNARFU; ISSN: 1355-8382
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**
SO RNA (2002), 8(8), 1056-1067
CODEN: RNARFU; ISSN: 1355-8382
AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel
AB The minus-strand genome of Sendai virus is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is assocd. with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC), whose hexamer phase is conserved within each virus group. To det. whether the

hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chem. modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepd. a matched set of viruses in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai viruses to edit their mRNAs in cell culture infections were examd., and the ability of DMS to chem. modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chem. modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is assocd. with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:351514 CAPLUS
DOCUMENT NUMBER: 137:90675
TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein
AUTHOR(S): Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
CORPORATE SOURCE: Department of Clinical and Experimental Medicine, Division of Microbiology, St Orsola General Hospital, University of Bologna, Bologna, 40138, Italy
SOURCE: Journal of General Virology (2002), 83(5), 1005-1012 CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein

SO Journal of General Virology (2002), 83(5), 1005-1012 CODEN: JGVIAY; ISSN: 0022-1317

AU Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.

AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. Here, the expression and localization of the UL53 gene product was analyzed. The results showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labeled, whereas **nucleocapsid** formations within the intranuclear skein are neg. Furthermore, the mature virus particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homolog in herpes simplex virus type 1.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE
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L2 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:56543 CAPLUS
DOCUMENT NUMBER: 126:101682
TITLE: Chimeric Sindbis viruses dependent on the NS3 protease
of hepatitis C virus
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, 00040, Italy
SOURCE: Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C virus
SO Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
AB The hepatitis C virus (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for anal. of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temp. dependence in the formation of lysis plaques yet stably expressed NS3.

L2 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:626027 CAPLUS
DOCUMENT NUMBER: 125:267435
TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length
AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel
CORPORATE SOURCE: Dep. Genetics Microbiology, Univ. Geneva Sch. Med., Geneva, Switz.
SOURCE: RNA (1996), 2(10), 1033-1045

CODEN: RNARFU; ISSN: 1355-8382
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Paramyxovirus RNA editing and the requirement for hexamer genome length
SO RNA (1996), 2(10), 1033-1045
CODEN: RNARFU; ISSN: 1355-8382
AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel
AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is assocd. with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examd. the effect of varying total genome length on the frequency and no. of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogs. We found that, as long as the purine run sequence and the region immediately upstream were unaltered, editing occurred during mRNA synthesis independent of the precise length of the mini-genome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase slippage during genome replication, and that this site serves the dual and complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L2 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:361797 CAPLUS
DOCUMENT NUMBER: 122:209332
TITLE: A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein
AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.
CORPORATE SOURCE: Dep. of Mol. Microbiology, Washington Univ. School of Medicine, St. Louis, MO, 63110-1073, USA
SOURCE: Virology (1995), 206(2), 1027-34
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
TI A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein
SO Virology (1995), 206(2), 1027-34
CODEN: VIRLAX; ISSN: 0042-6822
AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.

AB Most site-directed mutations in the gene encoding the small, membrane-assocd. 6K protein of Sindbis virus interfere selectively with virus assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids** . A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic anal. of this revertant showed that 2 addnl. mutations, which were mapped to the ectodomain of the E2 virus glycoprotein, were present in addn. to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal cells. Five new virus genetic constructs were prepd. by insertion of these mutations into the wild-type virus. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the virus. These results indicate that 6K may play an indirect role in the packing of the virus spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L2 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:507301 CAPLUS
DOCUMENT NUMBER: 115:107301
TITLE: Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses
AUTHOR(S): Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.
CORPORATE SOURCE: Sch. Med., Univ. South. California, Los Angeles, CA, 90033, USA
SOURCE: Virology (1991), 183(2), 647-57
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses
SO Virology (1991), 183(2), 647-57
CODEN: VIRLAX; ISSN: 0042-6822
AU Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.
AB The hemagglutinin-esterase (HE) membrane glycoprotein is present only in some members of the coronavirus family, including some strains of mouse hepatitis virus (MHV). In the JHM strain of MHV, expression of the HE gene is variable and corresponds to the no. of copies of a UCUAA pentanucleotide sequence present at the 3'-end of the leader RNA. This copy no. varies among MHV strains, depending on their passage history. The JHM isolates with 2 copies of UCUAA in their leader RNA showed a high level of HE expression, whereas the JHM isolate with 3 copies had a low-level expression. In this study, the anal. of HE gene expression was extended to other MHV strains. The synthesis of HE mRNA in these viruses also correlates with the copy no. of UCUAA in the leader RNA and the particular intergenic sequence preceding the HE gene. In 1 MHV strain, MHV-1, no detectable HE mRNA was synthesized, despite the presence of a proper transcription initiation signal. This lack of HE mRNA expression was consistent with a leader RNA contg. three UCUAA copies. However, mutations and deletions within the coding region of the MHV-1 HE gene have generated a stretch of sequence which resembled the transcriptional initiation motif, and was shown to initiate the synthesis of a novel smaller mRNA. These findings strengthened the theory that interactions

between leader RNA and transcriptional initiation sequences regulate MHV subgenomic mRNA transcription. Sequence anal. revealed that most MHV strains, through extensive mutations, deletions, or insertions, have lost the complete HE open reading frame, thus turning HE into a pseudogene. This high degree of variation is unusual as the other three structural proteins (spike, membrane, and **nucleocapsid**) are well-maintained. In contrast to bovine coronavirus, which apparently requires HE for viral replication, the HE protein in MHV may be only an accessory protein which is not necessary for viral replication. JHM and MHV-S, however, have preserved the expression of HE protein.

L2 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:472042 BIOSIS
DOCUMENT NUMBER: PREV200200472042
TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**.
AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)
CORPORATE SOURCE: (1) Department of Genetics and Microbiology, University of Geneva School of Medicine, CMU, 9 Ave de Champel, CH1211, Geneva: Daniel.Kolakofsky@Medecine.unige.ch Switzerland
SOURCE: RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067. http://uk.cambridge.org/journals/rna/. print. ISSN: 1355-8382.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**.
SO RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067. http://uk.cambridge.org/journals/rna/. print. ISSN: 1355-8382.
AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)
AB The minus-strand genome of Sendai virus is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is associated with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC), whose hexamer phase is conserved within each virus group. To determine whether the hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chemical modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepared a matched set of viruses in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai viruses to edit their mRNAs in cell culture infections were examined, and the ability of DMS to chemically modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chemical modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is

associated with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

L2 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:345323 BIOSIS
DOCUMENT NUMBER: PREV200200345323
TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.
AUTHOR(S): Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
CORPORATE SOURCE: (1) Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, St Orsola General Hospital, Via Massarenti 9, 40138, Bologna: dalmonte@med.unibo.it Italy
SOURCE: Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. http://vir.sgmjournals.org. print. ISSN: 0022-1317.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.
SO Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. http://vir.sgmjournals.org. print. ISSN: 0022-1317.
AU Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. In this work, the expression and localization of the UL53 gene product was analysed. Results obtained showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labelled, whereas **nucleocapsid** formations within the intranuclear skein are negative. Furthermore, the mature virus particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homologue in herpes simplex virus type 1.

L2 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:81269 BIOSIS
DOCUMENT NUMBER: PREV199799387972
TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C virus.
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P. Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy
SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427. ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C

virus.
SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.
ISSN: 0022-538X.
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
AB The hepatitis C virus (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for analysis of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

L2 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:524119 BIOSIS
DOCUMENT NUMBER: PREV199699246475
TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length.
AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)
CORPORATE SOURCE: (1) Dep. Genet. Microbiol., Univ. Geneva Sch. Med., CMU, 9 Ave de Champel, CH 1211 Geneva Switzerland
SOURCE: RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045.
ISSN: 1355-8382.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Paramyxovirus RNA editing and the requirement for hexamer genome length.
SO RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045.
ISSN: 1355-8382.
AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)
AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is associated with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examined the effect of varying total genome length on the frequency and number of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogues. We found that, as long as the purine run sequence and the region immediately upstream were

unaltered, editing occurred during mRNA synthesis independent of the precise length of the minigenome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase slippage during genome replication, and that this site serves the dual and complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L2 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:156639 BIOSIS
DOCUMENT NUMBER: PREV199598170939
TITLE: A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.
AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)
CORPORATE SOURCE: (1) Dep. Mol. Microbiol., Box 8230, Washington Univ. Sch. Med., St. Louis, MO 63110-1073 USA
SOURCE: Virology, (1995) Vol. 206, No. 2, pp. 1027-1034. ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
TI A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.
SO Virology, (1995) Vol. 206, No. 2, pp. 1027-1034. ISSN: 0042-6822.
AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)
AB Most site-directed mutations in the gene encoding the small, membrane-associated 6K protein of Sindbis virus interfere selectively with virus assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic analysis of this revertant showed that two additional mutations, which were mapped to the ectodomain of the E2 virus glycoprotein, were present in addition to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal calls. Five new virus genetic constructs were prepared by insertion of these mutations into the wild-type virus. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the virus. These results indicate that 6K may play an indirect role in the packing of the virus spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L2 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:327067 BIOSIS

DOCUMENT NUMBER: PREV199396035417
TITLE: Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV.
AUTHOR(S): Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)
CORPORATE SOURCE: (1) Dep. Biol., P.O. Box 4010, Georgia State Univ., Atlanta, GA 30302 USA
SOURCE: Virology, (1993) Vol. 194, No. 2, pp. 585-596. ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English

TI Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV.

SO Virology, (1993) Vol. 194, No. 2, pp. 585-596. ISSN: 0042-6822.

AU Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)

AB The apparently complete sequence of the RNA genome of the neurovirulent isolate of lactate dehydrogenase-elevating virus (LDV-C) has been determined. The LDV-C genome is at least 14,222 nucleotides in length and contains eight open reading frames (ORFs). ORF 1a, which encodes a protein of 242.8 kDa and is located at the 5' end of the genome, contains at least two putative papain-like cysteine protease domains, and one putative chymotrypsin-like serine protease domain. This ORF terminates with a UAG stop codon that can be bypassed if a -1 frameshift occurs. The frameshift region consists of a heptanucleotide "slippery" sequence, 5'-UUUAAAC-3', followed by a putative **pseudo**-knot. ORF 1b encodes a protein of 155.4 kDa containing, in its N-terminal portion, an RNA-dependent RNA polymerase and an RNA helicase domain separated by a Zn finger domain. Another domain of unknown function that is also conserved in coronaviruses and toroviruses is located at the C-terminus of the ORF 1b product. Three cleavage sites in the ORF 1a polyprotein and three in the ORF 1b polyprotein were predicted for the chymotrypsin-like protease and tentatively delimit the mature nonstructural proteins of LDV. Six small, overlapping 3' ORFs (ORFs 2 through 7) encode proteins with calculated sizes of 25.8, 21.6, 19.8, 23.9, 18.9, and 12.3 kDa. ORF 7 encodes the virion **nucleocapsid** protein Vp-1, while ORF 6 encodes the nonglycosylated envelope protein Vp2. ORFs 5, 4, 3, and 2 each encode glycoproteins which may be virion envelope proteins. LDV is closely related to equine arteritis virus, Lelystad virus (LV), and simian hemorrhagic fever virus. These four viruses belong to a new group of positive-strand RNA viruses and are related to coronaviruses and toroviruses.

=> D L8 IBIB TI SO AU ABS 1-12

L8 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:652537 CAPLUS
TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai **virus nucleocapsids**
AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel
CORPORATE SOURCE: Department of Genetics and Microbiology, University of

Geneva School of Medicine, Centre Medicale Universitaire, Geneva, CH1211, Switz.
SOURCE: RNA (2002), 8(8), 1056-1067
CODEN: RNARFU; ISSN: 1355-8382
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai **virus** **nucleocapsids**
SO RNA (2002), 8(8), 1056-1067
CODEN: RNARFU; ISSN: 1355-8382
AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel
AB The minus-strand genome of Sendai **virus** is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is assocd. with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC), whose hexamer phase is conserved within each **virus** group. To det. whether the hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chem. modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepd. a matched set of **viruses** in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai **viruses** to edit their mRNAs in cell culture infections were examd., and the ability of DMS to chem. modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chem. modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is assocd. with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:351514 CAPLUS
DOCUMENT NUMBER: 137:90675
TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein
AUTHOR(S): Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
CORPORATE SOURCE: Department of Clinical and Experimental Medicine, Division of Microbiology, St Orsola General Hospital, University of Bologna, Bologna, 40138, Italy
SOURCE: Journal of General Virology (2002), 83(5), 1005-1012

CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein
SO Journal of General Virology (2002), 83(5), 1005-1012
CODEN: JGVIAY; ISSN: 0022-1317
AU Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. Here, the expression and localization of the UL53 gene product was analyzed. The results showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labeled, whereas **nucleocapsid** formations within the intranuclear skein are neg. Furthermore, the mature **virus** particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homolog in herpes simplex **virus** type 1.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:56543 CAPLUS
DOCUMENT NUMBER: 126:101682
TITLE: Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, 00040, Italy
SOURCE: Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**
SO Journal of Virology (1997), 71(2), 1417-1427
CODEN: JOVIAM; ISSN: 0022-538X
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni
AB The hepatitis C **virus** (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block **virus** replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for anal. of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis **viruses** which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV

sequences were fused to the gene coding for the Sindbis **virus** structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious **viruses** in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis **virus**. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis **virus** portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious **viruses** which replicated at a lower rate than the parental construct and displayed a marked temp. dependence in the formation of lysis plaques yet stably expressed NS3.

L8 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:626027 CAPLUS
DOCUMENT NUMBER: 125:267435
TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length
AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel
CORPORATE SOURCE: Dep. Genetics Microbiology, Univ. Geneva Sch. Med., Geneva, Switz.
SOURCE: RNA (1996), 2(10), 1033-1045
CODEN: RNARFU; ISSN: 1355-8382
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Paramyxovirus RNA editing and the requirement for hexamer genome length
SO RNA (1996), 2(10), 1033-1045
CODEN: RNARFU; ISSN: 1355-8382
AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel
AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is assocd. with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examd. the effect of varying total genome length on the frequency and no. of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogs. We found that, as long as the purine run sequence and the region immediately upstream were unaltered, editing occurred during mRNA synthesis independent of the precise length of the mini-genome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase

slippage during genome replication, and that this site serves the dual and
complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L8 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:361797 CAPLUS
DOCUMENT NUMBER: 122:209332
TITLE: A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein
AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.
CORPORATE SOURCE: Dep. of Mol. Microbiology, Washington Univ. School of Medicine, St. Louis, MO, 63110-1073, USA
SOURCE: Virology (1995), 206(2), 1027-34
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
TI A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein
SO Virology (1995), 206(2), 1027-34
CODEN: VIRLAX; ISSN: 0042-6822
AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.
AB Most site-directed mutations in the gene encoding the small, membrane-assocd. 6K protein of Sindbis **virus** interfere selectively with **virus** assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic anal. of this revertant showed that 2 addnl. mutations, which were mapped to the ectodomain of the E2 **virus** glycoprotein, were present in addn. to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal cells. Five new **virus** genetic constructs were prepd. by insertion of these mutations into the wild-type **virus**. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the **virus**. These results indicate that 6K may play an indirect role in the packing of the **virus** spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L8 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:507301 CAPLUS
DOCUMENT NUMBER: 115:107301
TITLE: Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses
AUTHOR(S): Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.
CORPORATE SOURCE: Sch. Med., Univ. South. California, Los Angeles, CA,

90033, USA
SOURCE: Virology (1991), 183(2), 647-57
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses

SO Virology (1991), 183(2), 647-57
CODEN: VIRLAX; ISSN: 0042-6822

AU Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.

AB The hemagglutinin-esterase (HE) membrane glycoprotein is present only in some members of the coronavirus family, including some strains of mouse hepatitis **virus** (MHV). In the JHM strain of MHV, expression of the HE gene is variable and corresponds to the no. of copies of a UCUAA pentanucleotide sequence present at the 3'-end of the leader RNA. This copy no. varies among MHV strains, depending on their passage history. The JHM isolates with 2 copies of UCUAA in their leader RNA showed a high level of HE expression, whereas the JHM isolate with 3 copies had a low-level expression. In this study, the anal. of HE gene expression was extended to other MHV strains. The synthesis of HE mRNA in these **viruses** also correlates with the copy no. of UCUAA in the leader RNA and the particular intergenic sequence preceding the HE gene. In 1 MHV strain, MHV-1, no detectable HE mRNA was synthesized, despite the presence of a proper transcription initiation signal. This lack of HE mRNA expression was consistent with a leader RNA contg. three UCUAA copies. However, mutations and deletions within the coding region of the MHV-1 HE gene have generated a stretch of sequence which resembled the transcriptional initiation motif, and was shown to initiate the synthesis of a novel smaller mRNA. These findings strengthened the theory that interactions between leader RNA and transcriptional initiation sequences regulate MHV subgenomic mRNA transcription. Sequence anal. revealed that most MHV strains, through extensive mutations, deletions, or insertions, have lost the complete HE open reading frame, thus turning HE into a pseudogene. This high degree of variation is unusual as the other three structural proteins (spike, membrane, and **nucleocapsid**) are well-maintained. In contrast to bovine coronavirus, which apparently requires HE for viral replication, the HE protein in MHV may be only an accessory protein which is not necessary for viral replication. JHM and MHV-S, however, have preserved the expression of HE protein.

L8 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:472042 BIOSIS
DOCUMENT NUMBER: PREV200200472042
TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai **virus nucleocapsids**.
AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq,
Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)
CORPORATE SOURCE: (1) Department of Genetics and Microbiology, University of Geneva School of Medicine, CMU, 9 Ave de Champel, CH1211, Geneva: Daniel.Kolakofsky@Medecine.unige.ch Switzerland
SOURCE: RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067. http://uk.cambridge.org/journals/rna/. print. ISSN: 1355-8382.
DOCUMENT TYPE: Article
LANGUAGE: English

TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai **virus nucleocapsids**.

SO RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067.
http://uk.cambridge.org/journals/rna/. print.
ISSN: 1355-8382.
AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste;
Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)
AB The minus-strand genome of Sendai **virus** is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is associated with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC), whose
hexamer phase is conserved within each **virus** group. To determine whether the hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chemical modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepared a matched set of **viruses** in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai **viruses** to edit their mRNAs in cell culture infections were examined, and the ability of DMS to chemically modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chemical modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is associated with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

L8 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:345323 BIOSIS
DOCUMENT NUMBER: PREV200200345323
TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.
AUTHOR(S): Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
CORPORATE SOURCE: (1) Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, St Orsola General Hospital, Via Massarenti 9, 40138, Bologna: dalmonte@med.unibo.it Italy
SOURCE: Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. http://vir.sgmjournals.org. print.
ISSN: 0022-1317.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.
SO Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012.
http://vir.sgmjournals.org. print.
ISSN: 0022-1317.
AU Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.
AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. In this work, the expression and localization of the UL53 gene product was analysed. Results obtained showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in

cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labelled, whereas **nucleocapsid** formations within the intranuclear skein are negative. Furthermore, the mature **virus** particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homologue in herpes simplex **virus** type 1.

L8 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:81269 BIOSIS
DOCUMENT NUMBER: PREV199799387972
TITLE: Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**.
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P. Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy
SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**.
SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.
ISSN: 0022-538X.
AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)
AB The hepatitis C **virus** (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block **virus** replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for analysis of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis **viruses** which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis **virus** structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious **viruses** in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis **virus**. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis **virus** portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious **viruses** which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

L8 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1996:524119 BIOSIS
DOCUMENT NUMBER: PREV199699246475
TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length.
AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)
CORPORATE SOURCE: (1) Dep. Genet. Microbiol., Univ. Geneva Sch. Med., CMU, 9 Ave de Champel, CH 1211 Geneva Switzerland
SOURCE: RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045. ISSN: 1355-8382.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Paramyxovirus RNA editing and the requirement for hexamer genome length.
SO RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045.
ISSN: 1355-8382.
AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)
AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is associated with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examined the effect of varying total genome length on the frequency and number of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogues. We found that, as long as the purine run sequence and the region immediately upstream were unaltered, editing occurred during mRNA synthesis independent of the precise length of the minigenome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase slippage during genome replication, and that this site serves the dual and complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L8 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1995:156639 BIOSIS
DOCUMENT NUMBER: PREV199598170939
TITLE: A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.
AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)
CORPORATE SOURCE: (1) Dep. Mol. Microbiol., Box 8230, Washington Univ. Sch. Med., St. Louis, MO 63110-1073 USA
SOURCE: Virology, (1995) Vol. 206, No. 2, pp. 1027-1034. ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
TI A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new

mutations that map to the ectodomain of the E2 glycoprotein.
SO Virology, (1995) Vol. 206, No. 2, pp. 1027-1034.
ISSN: 0042-6822.
AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)
AB Most site-directed mutations in the gene encoding the small, membrane-associated 6K protein of Sindbis **virus** interfere selectively with **virus** assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic analysis of this revertant showed that two additional mutations, which were mapped to the ectodomain of the E2 **virus** glycoprotein, were present in addition to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal calls. Five new **virus** genetic constructs were prepared by insertion of these mutations into the wild-type **virus**. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the **virus**. These results indicate that 6K may play an indirect role in the packing of the **virus** spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L8 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1993:327067 BIOSIS
DOCUMENT NUMBER: PREV199396035417
TITLE: Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating **virus** (LDV.
AUTHOR(S): Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)
CORPORATE SOURCE: (1) Dep. Biol., P.O. Box 4010, Georgia State Univ., Atlanta, GA 30302 USA
SOURCE: Virology, (1993) Vol. 194, No. 2, pp. 585-596.
ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
TI Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating **virus** (LDV.
SO Virology, (1993) Vol. 194, No. 2, pp. 585-596.
ISSN: 0042-6822.
AU Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)
AB The apparently complete sequence of the RNA genome of the neurovirulent isolate of lactate dehydrogenase-elevating **virus** (LDV-C) has been determined. The LDV-C genome is at least 14,222 nucleotides in length
and contains eight open reading frames (ORFs). ORF 1a, which encodes a protein of 242.8 kDa and is located at the 5' end of the genome, contains at least two putative papain-like cysteine protease domains, and one putative chymotrypsin-like serine protease domain. This ORF terminates with a UAG stop codon that can be bypassed if a -1 frameshift occurs. The frameshift region consists of a heptanucleotide "slippery" sequence, 5'-UUUAAAC-3', followed by a putative **pseudo**-knot. ORF 1b encodes a protein of 155.4 kDa containing, in its N-terminal portion, an RNA-dependent RNA polymerase and an RNA helicase domain separated by a Zn finger domain. Another domain of unknown function that is also conserved in coronaviruses and toroviruses is located at the C-terminus of the ORF

1b product. Three cleavage sites in the ORF 1a polyprotein and three in the ORF 1b polyprotein were predicted for the chymotrypsin-like protease and tentatively delimit the mature nonstructural proteins of LDV. Six small, overlapping 3' ORFs (ORFs 2 through 7) encode proteins with calculated sizes of 25.8, 21.6, 19.8, 23.9, 18.9, and 12.3 kDa. ORF 7 encodes the virion **nucleocapsid** protein Vp-1, while ORF 6 encodes the nonglycosylated envelope protein Vp2. ORFs 5, 4, 3, and 2 each encode glycoproteins which may be virion envelope proteins. LDV is closely related to equine arteritis **virus**, Lelystad **virus** (LV), and simian hemorrhagic fever **virus**. These four **viruses** belong to a new group of positive-strand RNA **viruses** and are related to coronaviruses and toroviruses.

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 07:38:29 ON 31 OCT 2002

WEST Search History

DATE: Thursday, October 31, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ			
L6	tRNA and L4	11	L6
L5	tRNA and L4L4	0	L5
L4	L1 and L2	78	L4
L3	L1 and L2L2	0	L3
L2	viral adj capsid	903	L2
L1	(virus like particle)	741	L1

END OF SEARCH HISTORY

WEST Search History

DATE: Thursday, October 31, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ			
L9	capsid and L7	3	L9
L8	L7 and L2	0	L8
L7	crystalization	1518	L7
L6	tRNA and L4	11	L6
L5	tRNA and L4L4	0	L5
L4	L1 and L2	78	L4
L3	L1 and L2L2	0	L3
L2	viral adj capsid	903	L2
L1	(virus like particle)	741	L1

END OF SEARCH HISTORY

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=> " hepatitis C virus like particle"
         35919 "HEPATITIS"
       2959846 "C"
        274408 "VIRUS"
         52496 "VIRUSES"
        283713 "VIRUS"
                 ("VIRUS" OR "VIRUSES")
        541007 "LIKE"
           173 "LIKES"
        541156 "LIKE"
                 ("LIKE" OR "LIKES")
        551164 "PARTICLE"
        623424 "PARTICLES"
        938491 "PARTICLE"
                 ("PARTICLE" OR "PARTICLES")
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         51907 CORES
        256566 CORE
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         26759 TRNA
          5314 TRNAS
         27433 TRNA
                 (TRNA OR TRNAS)
L7           0 TRNA AND L5

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         26759 TRNA
          5314 TRNAS
         27433 TRNA
                 (TRNA OR TRNAS)
L8           0 TRNA AND L5

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           902 NUCLEOCAPSIDS
          4249 NUCLEOCAPSID
                 (NUCLEOCAPSID OR NUCLEOCAPSIDS)
L9           0 NUCLEOCAPSID AND L5

=> DIS L6 1- TI
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):N

=> DIS L6 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):Y
THE ESTIMATED COST FOR THIS REQUEST IS 6.87 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L6   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2002:248535  CAPLUS
DOCUMENT NUMBER:          136:398351
TITLE:                    Hepatitis C virus-
                          like particle morphogenesis
```

AUTHOR(S): Blanchard, Emmanuelle; Brand, Denys; Trassard, Sylvie;
Goudeau, Alain; Roingeard, Philippe
CORPORATE SOURCE: Laboratoire de Virologie, E3M-EA3250, IFR 82, Faculte de Medecine, Centre Hospitalier Universitaire, Tours, 37032, Fr.
SOURCE: Journal of Virology (2002), 76(8), 4073-4079
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Although much is known about the hepatitis C virus (HCV) genome, first cloned in 1989, little is known about HCV structure and assembly due to the lack of an efficient in vitro culture system for HCV. Using a recombinant Semliki forest virus replicon expressing genes encoding HCV structural proteins, we obsd. for the first time the assembly of these proteins into HCV-like particles in mammalian cells. This system opens up new possibilities for the investigation of viral morphogenesis and virus-host cell interactions.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:344513 CAPLUS
DOCUMENT NUMBER: 129:24164
TITLE: Synthesis and purification of **hepatitis C virus-like particles** from insect cells using a baculovirus vector
INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;
Liang, T. Jake; Baumert, Thomas F.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325

US 6387662 B1 20020514 US 1999-296441 19990421
PRIORITY APPLN. INFO.: US 1996-30238P P 19961108
WO 1997-US5096 W 19970325

AB Prodn. of enveloped RNA virus-like particles intracellularly in vitro in insect cells using a recombinant baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles contg. HCV **core** protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the purified HCV-like particles is disclosed.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:402184 CAPLUS
DOCUMENT NUMBER: 117:2184
TITLE: Non-A, Non-B hepatitis virus particle manufacture by expression of cloned cDNA
INVENTOR(S): Okayama, Hiroto; Fuke, Isao; Mori, Chisato; Takamizawa, Akihisa; Yoshida, Iwao
PATENT ASSIGNEE(S): Research Foundation for Microbial Diseases, Osaka University, Japan
SOURCE: Eur. Pat. Appl., 89 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 463848	A2	19920102	EP 1991-305717	19910625
EP 463848	A3	19920304		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 9168608	A1	19920102	AU 1991-68608	19910104
AU 650634	B2	19940630		
CN 1057861	A	19920115	CN 1991-100571	19910131
CA 2045326	AA	19911226	CA 1991-2045326	19910624
FI 9103068	A	19911226	FI 1991-3068	19910624
NO 9102459	A	19911227	NO 1991-2459	19910624
AU 9179256	A1	19920102	AU 1991-79256	19910624
AU 652941	B2	19940915		
CN 1059758	A	19920325	CN 1991-105293	19910625
HU 60773	A2	19921028	HU 1991-2118	19910625
JP 07258290	A2	19951009	JP 1991-180260	19910625
US 5747339	A	19980505	US 1994-324977	19941018
US 5847101	A	19981208	US 1995-384616	19950206
US 5998130	A	19991207	US 1997-904686	19970801
US 6217872	B1	20010417	US 1999-315850	19990521

PRIORITY APPLN. INFO.:
JP 1990-167466 A 19900625
JP 1990-230921 A 19900831
JP 1990-305605 A 19901109
US 1990-635451 A 19901228
JP 1991-132090 A 19910508
JP 1991-138493 A 19910514
US 1991-769996 B3 19911002
US 1993-99706 B1 19930730
US 1994-324977 A3 19941018
US 1997-904686 A3 19970801

AB CDNAs derived from a large segment of the hepatitis C virus genomic RNA

are cloned and expressed to yield viral particles suitable for use in vaccines and as diagnostic reagents. The particles include at least one antigen from the **core**, matrix, or envelope. Whole RNA was prepd. from plasma or liver of hepatitis C virus-infected patients, reverse transcribed and amplified by polymerase chain reaction by std. methods and cloned into .lambda.gt11 or pUC19. Banks were screened by immune screening with serum from infected patients. Individual clones were used to manuf. antigens in Escherichia coli. Individual cDNAs were ligated together to generate a single cDNA covering much of the viral genome. This cDNA was introduced into expression vectors for bacteria, yeast and animal cells. Human hepatocytes infected with a vaccinia virus-based expression vector carrying the cDNA yielded **hepatitis C virus-like particles** when obsd. in the electron microscope.

=> DIS L5 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):Y
THE ESTIMATED COST FOR THIS REQUEST IS 25.18 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L5 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:674085 CAPLUS
TITLE: Erratum: analysis of antigenicity and topology of E2 glycoprotein present on recombinant **hepatitis C virus-like particles**
AUTHOR(S): Clayton, Reginald F.; Owsianka, Ania; Aitken, Jim; Graham, Susan; Bhella, David; Patel, Arvind H.
CORPORATE SOURCE: MRC Virology Unit, Institute of Virology, University of Glasgow, Glasgow, G11 5JR, UK
SOURCE: Journal of Virology (2002), 76(18), 9562
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal; Errata
LANGUAGE: English
AB Unavailable

L5 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:674061 CAPLUS
TITLE: Interaction of **hepatitis C virus-like particles** and cells: a model system for studying viral binding and entry
AUTHOR(S): Triyatni, Miriam; Saunier, Bertrand; Maruvada, Padma; Davis, Anthony R.; Ulianich, Luca; Heller, Theo; Patel, Arvind; Kohn, Leonard D.; Liang, T. Jake
CORPORATE SOURCE: Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Journal of Virology (2002), 76(18), 9335-9344
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Hepatitis C virus-like particles** (HCV-LPs) contg. the structural proteins of HCV H77 strain (1a genotype) was used as a model for HCV virion to study

virus-cell interaction. HCV-LPs showed a buoyant d. of 1.17 to 1.22 g/cm3
in a sucrose gradient and formed double-shelled particles 35 to 49 nm in diam. Flow cytometry anal. by an indirect method (detection with anti-E2 antibody) and a direct method (use of dye-labeled HCV-LPs) showed that HCV-LPs binds to several human hepatic (primary hepatocytes, HepG2, HuH7, and NKNT-3) and T-cell (Molt-4) lines. HCV-LPs binding to cells occurred in a dose- and calcium-dependent manner and was not mediated by CD81. Scatchard plot anal. suggests the presence of two binding sites for HCV-LPs with high (Kd .apprx.1 .mu.g/mL) and low (Kd .apprx.50 to 60 .mu.g/mL) affinities of binding. Anti-E1 and -E2 antibodies inhibited HCV-LPs binding to cells. While preincubation of HCV-LPs with very-low-d.
lipoprotein (VLDL), low-d. lipoprotein (LDL), or high-d. lipoprotein (HDL)
blocked its binding to cells, preincubation of cells with VLDL, LDL, HDL, or anti-LDL-R antibody did not. Confocal microscopy anal. showed that, after binding to cells, dye-labeled HCV-LPs were internalized into the cytoplasm. This process could be inhibited with anti-E1 or anti-E2 antibodies, suggesting that E1 and E2 proteins mediate HCV-LPs binding and, subsequently, their entry into cells. Altogether, our results indicate that HCV-LPs can be used to further characterize the mechanisms involved in the early steps of HCV infection.
REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:536918 CAPLUS
DOCUMENT NUMBER: 137:244389
TITLE: Analysis of antigenicity and topology of E2 glycoprotein present on recombinant **hepatitis C virus-like particles**
AUTHOR(S): Clayton, Reginald F.; Owsianka, Ania; Aitken, Jim; Graham, Susan; Bhella, David; Patel, Arvind H.
CORPORATE SOURCE: MRC Virology Unit, Institute of Virology, University of Glasgow, Glasgow, G11 5JR, UK
SOURCE: Journal of Virology (2002), 76(15), 7672-7682
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Purifn. of hepatitis C virus (HCV) from sera of infected patients has proven elusive, hampering efforts to perform structure-function anal. of the viral components. Recombinant forms of the viral glycoproteins have been used instead for functional studies, but uncertainty exists as to whether they closely mimic the virion proteins. Here, we used HCV virus-like particles (VLPs) generated in insect cells infected with a recombinant baculovirus expressing viral structural proteins. Electron microscopic anal. revealed a population of pleomorphic VLPs that were at least partially enveloped with bilayer membranes and had viral glycoprotein spikes protruding from the surface. Immunogold labeling using specific monoclonal antibodies (MAbs) demonstrated these protrusions
to be the E1 and E2 glycoproteins. A panel of anti-E2 MAbs was used to probe the surface topol. of E2 on the VLPs and to compare the antigenicity
of the VLPs with that of truncated E2 (E2660) or the full-length (FL) E1E2

complex expressed in mammalian cells. While most MAbs bound to all forms of antigen, a no. of others showed striking differences in their abilities to recognize the various E2 forms. All MAbs directed against hypervariable region 1 (HVR-1) recognized both native and denatured E2660 with comparable affinities, but most bound either weakly or not at all to the FL E1E2 complex or to VLPs. HVR-1 on VLPs was accessible to these MAbs only after denaturation. Importantly, a subset of MAbs specific for amino acids 464 to 475 and 524 to 535 recognized E2660 but not VLPs or FL E1E2 complex. The antigenic differences between E2660, FL E1E2, and VLPs strongly point to the existence of structural differences, which may have functional relevance. Trypsin treatment of VLPs removed the N-terminal part of E2, resulting in a 42-kDa fragment. In the presence of detergent, this was further reduced to a trypsin-resistant 25-kDa fragment, which could be useful for structural studies.

REFERENCE COUNT: 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:496756 CAPLUS

DOCUMENT NUMBER: 137:123775

TITLE: Structural Features of Envelope Proteins on **Hepatitis C Virus-like Particles** as Determined by Anti-envelope Monoclonal Antibodies and CD81 Binding

AUTHOR(S): Triyatni, Miriam; Vergalla, John; Davis, Anthony R.; Hadlock, Kenneth G.; Foung, Steven K. H.; Liang, T. Jake

CORPORATE SOURCE: Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, 20892, USA

SOURCE: Virology (2002), 298(1), 124-132
CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The envelope glycoprotein E2 of hepatitis C virus (HCV) is a major component of the viral envelope. Knowledge of its topol. features and antigenic determinants in virions is crucial in understanding the viral binding sites to cellular receptor(s) and the induction of neutralizing antibodies. The lack of a robust cell culture system for virus propagation has hampered the characterization of E2 presented on the virion. Here the authors report the structural features of **hepatitis C virus-like particles** (HCV-LPs) of the 1a and 1b genotypes as detd. by various mouse and human monoclonal anti-envelope antibodies. Our results show that the E2 protein of HCV-LPs reacts with human monoclonal antibodies recognizing conformational determinants. Monoclonal antibodies (mAbs) specific for the hypervariable region 1 (HVR-1) sequence reacted strongly with HCV-LPs, suggesting that the HVR-1 is exposed on the viral surface. Several mAbs recognized both HCV-LPs with equally high affinity, indicating that the corresponding epitopes [amino acids (aa) 192-217 of E1 and aa 412-423, aa 522-531, and aa 640-653 of E2] are conserved in both genotypes and exposed on the surface of the HCV-LP. The E2 and E1/E2 dimers of 1a bound strongly to the recombinant large extracellular loop

(LEL) of CD81 (CD81-LEL) of human and African green monkey, while the HCV-LP of 1a bound weakly to human CD81-LEL. E1/E2 dimers and the HCV-LPs of 1b did not bind CD81-LEL, consistent with the notion that CD81 recognition by E2 is strain-specific and does not correlate with permissiveness of infection. A model of the topol. and exposed antigenic determinants of the envelope proteins of HCV is proposed.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:248535 CAPLUS
DOCUMENT NUMBER: 136:398351
TITLE: **Hepatitis C virus-like particle** morphogenesis
AUTHOR(S): Blanchard, Emmanuelle; Brand, Denys; Trassard, Sylvie; Goudeau, Alain; Roingeard, Philippe
CORPORATE SOURCE: Laboratoire de Virologie, E3M-EA3250, IFR 82, Faculte de Medecine, Centre Hospitalier Universitaire, Tours, 37032, Fr.
SOURCE: Journal of Virology (2002), 76(8), 4073-4079
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Although much is known about the hepatitis C virus (HCV) genome, first cloned in 1989, little is known about HCV structure and assembly due to the lack of an efficient in vitro culture system for HCV. Using a recombinant Semliki forest virus replicon expressing genes encoding HCV structural proteins, we obsd. for the first time the assembly of these proteins into HCV-like particles in mammalian cells. This system opens up new possibilities for the investigation of viral morphogenesis and virus-host cell interactions.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:79471 CAPLUS
DOCUMENT NUMBER: 136:260957
TITLE: Binding of **hepatitis C virus-like particles** derived from infectious clone H77C to defined human cell lines
AUTHOR(S): Wellnitz, Sabine; Klumpp, Bettina; Barth, Heidi; Ito, Susumu; Depla, Erik; Dubuisson, Jean; Blum, Hubert E.; Baumert, Thomas F.
CORPORATE SOURCE: Department of Medicine II, University of Freiburg, Freiburg, D-79106, Germany
SOURCE: Journal of Virology (2002), 76(3), 1181-1193
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Hepatitis C virus (HCV) is a leading cause of chronic hepatitis in the world. The study of viral entry and infection has been hampered by the inability to efficiently propagate the virus in cultured cells and the lack of a small-animal model. Recent studies have shown that in insect cells, the HCV structural proteins assemble into HCV-like particles (HCV-LPs) with morphol., biophys., and antigenic properties similar to those of putative virions isolated from HCV-infected humans. In this study, we used HCV-LPs derived from infectious clone H77C as a tool to examine virus-cell interactions. The binding of partially purified particles to human cell lines was analyzed by fluorescence-activated cell sorting with defined monoclonal antibodies to envelope glycoprotein E2. HCV-LPs demonstrated dose-dependent and saturable binding to defined human lymphoma and hepatoma cell lines but not to mouse cell lines. Binding could be inhibited by monoclonal anti-E2 antibodies, indicating that the HCV-LP-cell interaction was mediated by envelope glycoprotein E2. Binding appeared to be CD81 independent and did not correlate with low-d. lipoprotein receptor expression. Heat denaturation of HCV-LPs drastically reduced binding, indicating that the interaction of HCV-LPs with target cells was dependent on the proper conformation of the particles. In conclusion, our data demonstrate that insect cell-derived HCV-LPs bind specifically to defined human cell lines. Since the envelope proteins of HCV-LPs are presumably presented in a virion-like conformation, the binding of HCV-LPs to target cells may allow the study of virus-host cell interactions, including the isolation of HCV receptor candidates and antibody-mediated neutralization of binding.

REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:605968 CAPLUS
DOCUMENT NUMBER: 136:198509
TITLE: **Hepatitis C virus-like particles** induce virus-specific humoral and cellular immune responses in mice
AUTHOR(S): Lechmann, Martin; Murata, Kazumoto; Satoi, Jujin; Vergalla, John; Baumert, Thomas F.; Liang, T. Jake
CORPORATE SOURCE: Liver Diseases Section, NIDDK, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Hepatology (Philadelphia, PA, United States) (2001), 34(2), 417-423
CODEN: HPTLD9; ISSN: 0270-9139
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have recently described the prodn. of **hepatitis C virus-like particles** (HCV-LPs) in insect cells that resemble the putative virions. Here we evaluate the humoral and cellular immunogenicity of the virus-like particles with or without viral p7 protein, a small viral polypeptide that resides between the structural and nonstructural regions of the HCV polyprotein and whose function has not been defined. Immunized BALB/c mice developed high titers of anti-E2 antibodies and virus-specific cellular immune responses including cytotoxic T lymphocytes and T helper responses with gamma interferon prodn. The virus-like particles without p7 generated a higher cellular immune response with a more TH1 profile than the particles with p7.

Immunization of heat-denatured particles resulted in substantially lower humoral and cellular responses, suggesting that the immunogenicity is strongly dependent on particle formation. Administration of CpG oligonucleotide or cationic lipid 3.beta.-[N-(N',N'-dimethylaminoethane)carbamoyl]-cholesterol (DC-Chol), two potent adjuvants, did not significantly enhance the immunogenicity of HCV-LPs. These results indicate that HCV-LPs can induce humoral and cellular immune responses and offer a promising approach to vaccine development.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:396894 CAPLUS
DOCUMENT NUMBER: 135:18547
TITLE: Virus-like particles for vaccination against hepatitis
INVENTOR(S): Selby, Mark; Glazer, Edward; Houghton, Michael
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001038358	A2	20010531	WO 2000-US32249	20001122
WO 2001038358	A3	20020110		
WO 2001038358	C2	20020627		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1233783	A2	20020828	EP 2000-982225	20001122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 1999-167224P A2 19991124
WO 2000-US32249 W 20001122

AB The authors disclose the construction of chimeric antigens derived from hepatitis B virus (HBV) and hepatitis C virus (HCV) which form virus-like particles when co-expressed with an excess of hepatitis B virus surface antigen (HBsAg). The chimeric antigens are fusion proteins contg. an immunogenic peptide derived from HCV coupled to the N-terminus of HBsAg. Also described are nucleic acid constructs and vectors for transfection of cells and expression of the chimeric antigens. The authors also disclose methods for producing HBV/HCV virus-like particles contg. the chimeric antigens, cell lines for producing the virus-like particles, combination vaccines contg. the virus-like particles, and DNA vaccines that express the virus-like particles.

L5 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:659593 CAPLUS
DOCUMENT NUMBER: 134:221173
TITLE: Antibodies against **hepatitis C virus-like particles** and viral clearance in acute and chronic hepatitis C
AUTHOR(S): Baumert, Thomas F.; Wellnitz, Sabine; Aono, Shigeaki; Satoi, Jujin; Herion, David; Gerlach, Tilman; Pape, Gerd R.; Lau, Johnson Y. N.; Hoofnagle, Jay H.; Blum, Hubert E.; Liang, T. Jake
CORPORATE SOURCE: Department of Medicine II, University of Freiburg Medical School, Freiburg, D-79106, Germany
SOURCE: Hepatology (Philadelphia) (2000), 32(3), 610-617
CODEN: HPTLD9; ISSN: 0270-9139
PUBLISHER: W. B. Saunders Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors recently described the efficient assembly of hepatitis C virus (HCV) structural proteins into HCV-like particles (HCV-LPs) in insect cells. These noninfectious HCV-LPs have similar morphol. and biophys. properties as putative virions isolated from HCV-infected humans and can induce a broadly directed immune response in animal models. The HCV envelope proteins of HCV-LPs are presumably presented in a native, virion-like conformation and may therefore interact with anti-envelope antibodies directed against conformational epitopes. Here, HCV-LPs were used as capture antigens in an ELISA to detect and quantify antibodies against HCV structural proteins in patients with acute and chronic hepatitis C. High titers of anti-HCV-LP antibodies were detected in patients chronically infected with HCV genotypes 1-6. In contrast to individuals with chronic hepatitis C, patients with acute self-limited hepatitis C displayed only a transient and weak seroreactivity against HCV-LPs. Patients with chronic HCV infection successfully treated with interferon demonstrated a gradual decline of anti-HCV-LP titers during or subsequent to viral clearance. Sustained interferon responders were characterized by higher pretreatment levels of anti-HCV-LP antibodies as compared with nonresponders. Thus, HCV infection is assocd. with limited humoral immunity against the envelope proteins present on the HCV-LPs. An HCV-LP-based ELISA may be a useful diagnostic tool to distinguish acute hepatitis C from chronic HCV infection with exacerbation, and to predict viral clearance in response to interferon.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:344513 CAPLUS
DOCUMENT NUMBER: 129:24164
TITLE: Synthesis and purification of **hepatitis C virus-like particles** from insect cells using a baculovirus vector
INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;
Liang, T. Jake; Baumert, Thomas F.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325
US 6387662	B1	20020514	US 1999-296441	19990421

PRIORITY APPLN. INFO.: US 1996-30238P P 19961108
WO 1997-US5096 W 19970325

AB Prodn. of enveloped RNA virus-like particles intracellularly in vitro in insect cells using a recombinant baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the purified HCV-like particles is disclosed.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L12 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:478160 CAPLUS
DOCUMENT NUMBER: 121:78160
TITLE: Hepatitis C virus particle detected by immunoelectron microscopic study
AUTHOR(S): Kaito, Masahiko; Watanabe, Shozo; Tsukiyama-Kohara, Kyoko; Yamaguchi, Kenjiro; Kobayashi, Yoshinao; Konishi, Masayoshi; Yokoi, Masato; Ishida, Satoshi; Suzuki, Shiro; Kohara, Michinori
CORPORATE SOURCE: Sch. Med., Mie Univ., Mie, 514, Japan
SOURCE: Journal of General Virology (1994), 75(7), 1755-60
CODEN: JGVIAY; ISSN: 0022-1317
DOCUMENT TYPE: Journal
LANGUAGE: English
AB To clarify the morphol. of hepatitis C virus (HCV), an indirect immunogold electron microscope study was carried out on two plasma samples with high HCV RNA titers using polyclonal and monoclonal antibodies specific to the putative HCV envelope protein. Spherical **virus-like particles** 55 to 65 nm in diam. with spike-like projections, were found in 1.14 to 1.6 g/mL fractions after sucrose d. gradient centrifugation. These particles were found only in HCV-infected blood donors and had morphol. features similar to those of **flaviviruses**. Moreover, these particles specifically reacted with the polyclonal and monoclonal antibodies to the putative HCV envelope protein. This is the first known report in which the morphol. of the HCV particle is clearly shown.

L12 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:55842 CAPLUS
DOCUMENT NUMBER: 118:55842
TITLE: Regulation of the late events in **flavivirus** protein processing and maturation
AUTHOR(S): Yamshchikov, Vladimir F.; Compans, Richard W.
CORPORATE SOURCE: Dep. Microbiol., Univ. Alabama, Birmingham, AL, 35294, USA
SOURCE: Virology (1992), 192(1), 38-51
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In order to det. the requirements for secretion of **flavivirus** structural proteins, the authors analyzed the expression of several West Nile **flavivirus** gene cassettes of different lengths in vaccinia virus expression systems. Expression of the longest cassette coding for the 5'-nontranslated region, proteins C through NS2B, and the protease domain of NS3, resulted in secretion of prM-E complexes and cleavage of prM. The presence and proper processing of the NS2A-NS2B-NS3 region appeared to be necessary for prM-E secretion. These proteins were released from cells mostly as membranous complexes which may represent empty viral envelopes. Cleavage of the membrane-assocd. intracellular form of protein C (Ci) to produce the virion form (Ce) appeared to be crit. for release of viral proteins. The presence and proper cleavage of the NS2A-NS2B-NS3 region were also found to be necessary for efficient C-prM cleavage by signalases. The NS2B-NS3 complex was implicated in cleavage of the intracellular form of protein C. Formation of a low level of **virus-like particles** was detected by electron microscopy. A model for virion formation, suggesting a crit.

role of the NS2B and NS3 proteins, is discussed.

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THE ESTIMATED COST FOR THIS REQUEST IS 2.29 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:396894 CAPLUS
DOCUMENT NUMBER: 135:18547
TITLE: Virus-like particles for vaccination against hepatitis
INVENTOR(S): Selby, Mark; Glazer, Edward; Houghton, Michael
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001038358	A2	20010531	WO 2000-US32249	20001122
WO 2001038358	A3	20020110		
WO 2001038358	C2	20020627		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1233783	A2	20020828	EP 2000-982225	20001122
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: US 1999-167224P A2 19991124
WO 2000-US32249 W 20001122

AB The authors disclose the construction of chimeric antigens derived from hepatitis B virus (HBV) and hepatitis C virus (HCV) which form virus-like particles when co-expressed with an excess of hepatitis B virus surface antigen (HBsAg). The chimeric antigens are fusion proteins contg. an immunogenic peptide derived from HCV coupled to the N-terminus of HBsAg. Also described are nucleic acid constructs and vectors for transfection of cells and expression of the chimeric antigens. The authors also disclose methods for producing HBV/HCV virus-like particles contg. the chimeric antigens, cell lines for producing the virus-like particles, combination vaccines contg. the virus-like particles, and DNA vaccines that express the virus-like particles.

L5 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:402184 CAPLUS
DOCUMENT NUMBER: 117:2184
TITLE: Non-A, Non-B hepatitis virus particle manufacture by expression of cloned cDNA
INVENTOR(S): Okayama, Hiroto; Fuke, Isao; Mori, Chisato; Takamizawa, Akihisa; Yoshida, Iwao
PATENT ASSIGNEE(S): Research Foundation for Microbial Diseases, Osaka University, Japan
SOURCE: Eur. Pat. Appl., 89 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 463848	A2	19920102	EP 1991-305717	19910625
EP 463848	A3	19920304		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 9168608	A1	19920102	AU 1991-68608	19910104
AU 650634	B2	19940630		
CN 1057861	A	19920115	CN 1991-100571	19910131
CA 2045326	AA	19911226	CA 1991-2045326	19910624
FI 9103068	A	19911226	FI 1991-3068	19910624
NO 9102459	A	19911227	NO 1991-2459	19910624
AU 9179256	A1	19920102	AU 1991-79256	19910624
AU 652941	B2	19940915		
CN 1059758	A	19920325	CN 1991-105293	19910625
HU 60773	A2	19921028	HU 1991-2118	19910625
JP 07258290	A2	19951009	JP 1991-180260	19910625
US 5747339	A	19980505	US 1994-324977	19941018
US 5847101	A	19981208	US 1995-384616	19950206
US 5998130	A	19991207	US 1997-904686	19970801
US 6217872	B1	20010417	US 1999-315850	19990521

PRIORITY APPLN. INFO.:
JP 1990-167466 A 19900625
JP 1990-230921 A 19900831
JP 1990-305605 A 19901109
US 1990-635451 A 19901228
JP 1991-132090 A 19910508
JP 1991-138493 A 19910514
US 1991-769996 B3 19911002
US 1993-99706 B1 19930730
US 1994-324977 A3 19941018
US 1997-904686 A3 19970801

AB CDNAs derived from a large segment of the hepatitis C virus genomic RNA are cloned and expressed to yield viral particles suitable for use in vaccines and as diagnostic reagents. The particles include at least one antigen from the core, matrix, or envelope. Whole RNA was prepd. from plasma or liver of hepatitis C virus-infected patients, reverse transcribed and amplified by polymerase chain reaction by std. methods and

cloned into .lambda.gt11 or pUC19. Banks were screened by immune screening with serum from infected patients. Individual clones were used to manuf. antigens in Escherichia coli. Individual cDNAs were ligated together to generate a single cDNA covering much of the viral genome. This cDNA was introduced into expression vectors for bacteria, yeast and animal cells. Human hepatocytes infected with a vaccinia virus-based expression vector carrying the cDNA yielded **hepatitis C**

virus-like particles when obsd. in the electron microscope.

L12 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:344513 CAPLUS
DOCUMENT NUMBER: 129:24164
TITLE: Synthesis and purification of hepatitis C **virus-like particles** from insect cells using a baculovirus vector
INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;
Liang, T. Jake; Baumert, Thomas F.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325
US 6387662	B1	20020514	US 1999-296441	19990421

PRIORITY APPLN. INFO.: US 1996-30238P P 19961108
WO 1997-US5096 W 19970325

AB Prodn. of enveloped RNA **virus-like particles** intracellularly in vitro in insect cells using a recombinant baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the purified HCV-like particles is disclosed.
REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L12 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:494028 CAPLUS
DOCUMENT NUMBER: 127:202729
TITLE: Expression of the dengue virus structural proteins in Pichia pastoris leads to the generation of **virus-like particles**
AUTHOR(S): Sugrue, Richard Joseph; Fu, Jianlin; Howe, Josephine; Chan, Yow-Cheong
CORPORATE SOURCE: Dengue Virus Group, Institute of Molecular and Cell Biology, National University of Singapore, Singapore
SOURCE: Journal of General Virology (1997), 78(8), 1861-1866
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal
LANGUAGE: English

AB We have expressed cDNA encoding the dengue virus structural proteins in Pichia pastoris by chromosomal integration of an expression cassette contg. the dengue virus structural genes (CprME). The yeast recombinant E protein migrated during SDS-PAGE as a 65 kDa protein when analyzed by Western blotting and radioimmunopptn., which is the expected mol. mass for correctly processed and glycosylated E protein. Treatment with endoglycosidases showed that the recombinant E protein was modified by the addn. of short mannose chains. The E protein migrated with a buoyant d. of 1.13 g/cm3 when analyzed using sucrose d. gradient centrifugation. Spherical structures with an av. diam. of 30 nm, whose morphol. resembles dengue virions, were obsd. in the purified fractions using transmission electron microscopy. Furthermore, the **virus-like particles** were immunogenic in animals and were able to induce neutralizing antibodies. This is the first report that expression of the structural genes of a **flavivirus** in yeast is able to generate particulate structures that resemble virions.

L12 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:155646 CAPLUS
DOCUMENT NUMBER: 131:68778
TITLE: Noncytopathic **Flavivirus** Replicon RNA-Based System for Expression and Delivery of Heterologous Genes
AUTHOR(S): Varnavski, Andrei N.; Khromykh, Alexander A.
CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Brisbane, 4029, Australia
SOURCE: Virology (1999), 255(2), 366-375
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Noncytopathic replicons of the **flavivirus** Kunjin (KUN) were employed for expression and delivery of heterologous genes. Replicon vector C20DX2Arep, contg. a unique cloning site followed by the sequence of 2A autoprotease of foot-and-mouth disease virus, was constructed and used for expression of a no. of heterologous genes including chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), .beta.-galactosidase, glycoprotein G of vesicular stomatitis virus, and the Core and NS3 genes of hepatitis C virus. The expression and proper processing of these genes upon transfection of BHK21 cells with the recombinant replicon RNAs were demonstrated by immunofluorescence, radioimmunopptn., and appropriate reporter gene assays. Most of these recombinant KUN replicon RNAs were also successfully packaged into secreted **virus-like particles** (**VLPs**) by subsequent transfection with Semliki Forest virus replicon RNA expressing KUN structural genes. Infection of BHK21 and Vero cells with these **VLPs** resulted in continuous replication of the recombinant replicon RNAs and prolonged expression of the cloned genes without any cytopathic effect. We also developed a replicon vector for generation of stable cell lines continuously expressing heterologous genes by inserting an encephalomyelocarditis virus internal ribosomal entry site-neomycin transferase gene cassette into the 3'-untranslated region of the C20DX2Arep vector. Using this vector (C20DX2ArepNeo), stable BHK cell lines persistently expressing GFP and CAT genes for up to 17 passages were established. Thus noncytopathic KUN replicon vectors with the ability to be packaged into **VLPs** should provide a useful tool for the development of noninfectious and noncytopathic vaccines as well as for gene therapy applications. (c) 1999 Academic Press.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:384227 CAPLUS
DOCUMENT NUMBER: 133:29600
TITLE: Capsid particles of hepatitis B core antigen for presentation of immunogenic components
INVENTOR(S): Murray, Kenneth
PATENT ASSIGNEE(S): Biogen, Inc., USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032625	A1	20000608	WO 1999-US28755	19991203
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 9915942	A	20010821	BR 1999-15942	19991203
EP 1135408	A1	20010926	EP 1999-961935	19991203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002532387	T2	20021002	JP 2000-585266	19991203
US 2002064533	A1	20020530	US 2001-873459	20010604
NO 2001002760	A	20010806	NO 2001-2760	20010605

PRIORITY APPLN. INFO.: US 1998-110911P P 19981204
WO 1999-US28755 W 19991203

AB The authors discloses the use of hepatitis B virus (HBV) core antigen particles for presentation to the immune system of multiple immunogen specificities. The immunogens, epitopes, or other related structures, are crosslinked or fused to HBV capsid-binding peptides that selectively bind to HBV core protein. Mixts. of different immunogens and/or capsid-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or multivalent HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compns., as well as in diagnostic applications.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:55842 CAPLUS
DOCUMENT NUMBER: 118:55842
TITLE: Regulation of the late events in **flavivirus** protein processing and maturation
AUTHOR(S): Yamshchikov, Vladimir F.; Compans, Richard W.
CORPORATE SOURCE: Dep. Microbiol., Univ. Alabama, Birmingham, AL, 35294, USA
SOURCE: Virology (1992), 192(1), 38-51
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In order to det. the requirements for secretion of **flavivirus** structural proteins, the authors analyzed the expression of several West Nile **flavivirus** gene cassettes of different lengths in vaccinia virus expression systems. Expression of the longest cassette coding for the 5'-nontranslated region, proteins C through NS2B, and the protease domain of NS3, resulted in secretion of prM-E complexes and cleavage of prM. The presence and proper processing of the NS2A-NS2B-NS3 region appeared to be necessary for prM-E secretion. These proteins were released from cells mostly as membranous complexes which may represent empty viral envelopes. Cleavage of the membrane-assocd. intracellular form of protein C (Ci) to produce the virion form (Ce) appeared to be crit. for release of viral proteins. The presence and proper cleavage of the NS2A-NS2B-NS3 region were also found to be necessary for efficient C-prM cleavage by signalases. The NS2B-NS3 complex was implicated in cleavage of the intracellular form of protein C. Formation of a low level of **virus-like particles** was detected by electron microscopy. A model for virion formation, suggesting a crit. role of the NS2B and NS3 proteins, is discussed.

L13 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:384227 CAPLUS
DOCUMENT NUMBER: 133:29600
TITLE: **Capsid** particles of hepatitis B core antigen for presentation of immunogenic components
INVENTOR(S): Murray, Kenneth
PATENT ASSIGNEE(S): Biogen, Inc., USA
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032625	A1	20000608	WO 1999-US28755	19991203
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 9915942	A	20010821	BR 1999-15942	19991203
EP 1135408	A1	20010926	EP 1999-961935	19991203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002532387	T2	20021002	JP 2000-585266	19991203
US 2002064533	A1	20020530	US 2001-873459	20010604
NO 2001002760	A	20010806	NO 2001-2760	20010605

PRIORITY APPLN. INFO.: US 1998-110911P P 19981204
WO 1999-US28755 W 19991203

AB The authors discloses the use of hepatitis B virus (HBV) core antigen particles for presentation to the immune system of multiple immunogen specificities. The immunogens, epitopes, or other related structures, are crosslinked or fused to HBV **capsid**-binding peptides that selectively bind to HBV core protein. Mixts. of different immunogens and/or **capsid**-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or multivalent HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compns., as well as in diagnostic applications.

L16 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:155646 CAPLUS
DOCUMENT NUMBER: 131:68778
TITLE: Noncytopathic **Flavivirus** Replicon RNA-Based System for Expression and Delivery of Heterologous Genes
AUTHOR(S): Varnavski, Andrei N.; Khromykh, Alexander A.
CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Brisbane, 4029, Australia
SOURCE: Virology (1999), 255(2), 366-375
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Noncytopathic replicons of the **flavivirus** Kunjin (KUN) were employed for expression and delivery of heterologous genes. Replicon vector C20DX2Arep, contg. a unique cloning site followed by the sequence of 2A autoprotease of foot-and-mouth disease virus, was constructed and used for expression of a no. of heterologous genes including chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), .beta.-galactosidase, glycoprotein G of vesicular stomatitis virus, and the **Core** and NS3 genes of hepatitis C virus. The expression and proper processing of these genes upon transfection of BHK21 cells with the recombinant replicon RNAs were demonstrated by immunofluorescence, radioimmunopptn., and appropriate reporter gene assays. Most of these recombinant KUN replicon RNAs were also successfully packaged into secreted **virus-like particles** (**VLPs**) by subsequent transfection with Semliki Forest virus replicon RNA expressing KUN structural genes. Infection of BHK21 and Vero cells with these **VLPs** resulted in continuous replication of the recombinant replicon RNAs and prolonged expression of the cloned genes without any cytopathic effect. We also developed a replicon vector for generation of stable cell lines continuously expressing heterologous genes by inserting an encephalomyelocarditis virus internal ribosomal entry site-neomycin transferase gene cassette into the 3'-untranslated region of the C20DX2Arep vector. Using this vector (C20DX2ArepNeo), stable BHK cell lines persistently expressing GFP and CAT genes for up to 17 passages were established. Thus noncytopathic KUN replicon vectors with the ability to be packaged into **VLPs** should provide a useful tool for the development of noninfectious and noncytopathic vaccines as well as for gene therapy applications. (c) 1999 Academic Press.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/40, C07K 14/18, A61K 39/29, C07K 16/10, G01N 33/569, C12N 5/10

A1

(11) International Publication Number: **WO 98/21338**

(43) International Publication Date: 22 May 1998 (22.05.98)

(21) International Application Number: PCT/US97/05096

(22) International Filing Date: 25 March 1997 (25.03.97)

(30) Priority Data:
60/030,238 8 November 1996 (08.11.96) US

(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LIANG, T., Jake [US/US]; 9417 Lost Trail Way, Potomac, MD 20854 (US). BAUMERT, Thomas, F. [US/US]; Apartment 2, 4867 Battery Lane, Bethesda, MD 20814 (US).

(74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, 16th floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).

(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
With amended claims.

(54) Title: SYNTHESIS AND PURIFICATION OF HEPATITIS C VIRUS-LIKE PARTICLES

(57) Abstract

Production of enveloped RNA virus-like particles intracellularly *in vitro* in insect cells using a recombinant baculovirus vector containing a cDNA coding for viral structural proteins is disclosed. *In vitro* production and purification of hepatitis C virus (HCV)-like particles containing HCV core protein, E1 protein and E2 protein is disclosed. Production of antibodies *in vivo* to the purified HCV-like particles is disclosed.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/US 97/05096

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/40 C07K14/18 A61K39/29 C07K16/10 G01N33/569
C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEPATOLOGY, vol. 24 , no. 4,Pt.2(Supplement), October 1996, page 251A XP002036710 T.F.BAUMERT ET AL.: "Synthesis of Hepatitis C virus-like particles in insect cells" see abstract 500 ---	1-15, 19-22
X	EP 0 577 894 A (KOREA GREEN CROSS CORPORATION) 12 January 1994 see page 6, line 46 - page 8, line 26 --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1997	14.08.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/US 97/05096

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF GENERAL VIROLOGY, vol. 73, no. PART 07, 1 July 1992, pages 1819-1824, XP000288658 THOMSEN D R ET AL: "Expression of feline leukaemia virus gp85 and gag proteins and assembly into virus-like particles using the baculovirus expression vector system" see the whole document	1-5
X	WO 95 33053 A (CHIRON CORPORATION) 7 December 1995	16,17
A	see page 19, line 20 - line 27	10,11

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

:national application No.
PCT/US 97/05096

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 5 and 6 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte ɔnal Application No
PCT/US 97/05096

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 577894 A	12-01-94	JP 6321997 A	22-11-94
		US 5580773 A	03-12-96
		US 5443828 A	22-08-95
WO 9533053 A	07-12-95	AU 2550995 A	21-12-95
		CA 2190265 A	07-12-95
		EP 0760855 A	12-03-97

Form PCT/ISA/210 (patent family annex) (July 1992)

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=> "self-assembly"
        254992 "SELF"
            20 "SELFS"
            31 "SELVES"
        255037 "SELF"
                 ("SELF" OR "SELFS" OR "SELVES")
        101096 "ASSEMBLY"
         25117 "ASSEMBLIES"
        116983 "ASSEMBLY"
                 ("ASSEMBLY" OR "ASSEMBLIES")
L1       11719 "SELF-ASSEMBLY"
                 ("SELF"(W)"ASSEMBLY")

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            70 "VITROS"
        516972 " IN VITRO"
                 ("VITRO" OR "VITROS")
L2         422 " IN VITRO" AND L1

=> " cell free" and L1
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       1413425 "CELLS"
       2138443 "CELL"
                 ("CELL" OR "CELLS")
       1071709 "FREE"
           531 "FREES"
       1072153 "FREE"
                 ("FREE" OR "FREES")
         32767 " CELL FREE"
                 ("CELL"(W)"FREE")
L3          18 " CELL FREE" AND L1

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             5 "VIRALS"
        110279 "VIRAL"
                 ("VIRAL" OR "VIRALS")
        541007 "LIKE"
           173 "LIKES"
        541156 "LIKE"
                 ("LIKE" OR "LIKES")
            19 "PARTICEL"
            19 "PARTICELS"
            38 "PARTICEL"
                 ("PARTICEL" OR "PARTICELS")
             0 "VIRAL LIKE PARTICEL"
                 ("VIRAL"(W)"LIKE"(W)"PARTICEL")
L4           0 "VIRAL LIKE PARTICEL" AND L3

=> "virus like particle"
        274408 "VIRUS"
         52496 "VIRUSES"
        283713 "VIRUS"
                 ("VIRUS" OR "VIRUSES")
        541007 "LIKE"
           173 "LIKES"
        541156 "LIKE"
                 ("LIKE" OR "LIKES")
        551164 "PARTICLE"
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        623424 "PARTICLES"
        938491 "PARTICLE"
                 ("PARTICLE" OR "PARTICLES")
L5        1489 "VIRUS LIKE PARTICLE"
                 ("VIRUS"(W)"LIKE"(W)"PARTICLE")

=> L5 and L3
L6           2 L5 AND L3
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                                                           ENTRY    SESSION
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=> tRNA
L1       42320 TRNA

=> crystalization and L1
L2           0 CRYSTALIZATION AND L1

=> "Transfer RNA or tRNA"
L3         788 "TRANSFER RNA OR TRNA"

=> crystalization and L3
L4           0 CRYSTALIZATION AND L3

=> crystalization
L5         139 CRYSTALIZATION

=> :5 and L3
L6         224 :5 AND L3

=> L5 and L3
L7           0 L5 AND L3

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 12:57:34 ON 31 OCT 2002
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L3 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1988:626196 CAPLUS
DOCUMENT NUMBER: 109:226196
TITLE: In vitro assay for protein-protein interaction: carboxyl-terminal 40 residues of simian virus 40 structural protein VP3 contain a determinant for interaction with VP1
AUTHOR(S): Gharakhanian, Editte; Takahashi, Jeffrey; Clever, Jared; Kasamatsu, Harumi
CORPORATE SOURCE: Mol. Biol. Inst., Univ. California, Los Angeles, CA, 90024-1606, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1988), 85(18), 6607-11
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Intermol. interactions between polypeptide chains play essential roles in the functioning of proteins. An in vitro assay system for identifying and characterizing such interactions is described. Such interactions are difficult to study in vivo. Synthetic, nonmethyl-capped RNAs were translated in a **cell-free** protein-synthesizing system. The translation products were allowed to interact posttranslationally to form protein-protein complexes. The chem. nature of the protein interaction(s) was detd. by coimmunopptn. of assocg. proteins, sedimentation through sucrose gradients, followed by SDS-polyacrylamide gel electrophoresis or by nonreducing SDS-polyacrylamide gel electrophoresis. The system has been utilized to show the **self-assembly** of monomeric VP1, the major structural protein of simian virus 40, into disulfide-linked pentamers and to show the noncovalent interaction of another structural protein, VP3, with VP1 at low monomer concns. Addnl., the carboxyl-terminal 40 amino acids of VP3 are shown to be essential and sufficient for its interaction with VP1 in vitro. The in vitro assay system described here provides a method for identifying the domains involved in, and the mol. nature of, protein-protein interactions, which play an important role in such biol. phenomena as replication, transcription, translation, transport, ligand binding, and assembly.

L3 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:3546 CAPLUS
DOCUMENT NUMBER: 118:3546
TITLE: Expression, **self-assembly**, and antigenicity of the Norwalk virus capsid protein
AUTHOR(S): Jiang, Xi; Wang, Min; Graham, David Y.; Estes, Mary K.
CORPORATE SOURCE: Div. Mol. Virol., Baylor Coll. Med., Houston, TX, 77030, USA
SOURCE: Journal of Virology (1992), 66(11), 6527-32
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Norwalk virus capsid protein was produced by expression of the 2nd and 3rd open reading frames of the Norwalk virus genome, using a **cell-free** translation system and baculovirus recombinants. Anal. of the expressed products showed that the 2nd open reading frame encodes a protein with an apparent mol. wt. of 58,000 (58K protein) and that this protein self-assembles to form empty virus-like particles similar to native capsids in size and appearance. The antigenicity of these particles was demonstrated by immunopptn. and ELISA of paired serum samples from volunteers who developed illness following Norwalk virus challenge. These particles also induced high levels of Norwalk virus-specific serum antibody in lab. animals following parenteral inoculation. A minor 34K protein was also found in infected insect cells.

Amino acid sequence anal. of the N terminus of the 34K protein indicated that the 34K protein was a cleavage product of the 58K protein. The availability of large amts. of recombinant Norwalk virus particles will allow the development of rapid, sensitive, and reliable tests for the diagnosis of Norwalk virus infection as well as the implementation of structural studies.

L3 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:735794 CAPLUS
DOCUMENT NUMBER: 126:17546
TITLE: **Self-assembly** of in vitro-translated human papillomavirus type 16 L1 capsid protein into virus-like particles and antigenic reactivity of the protein
AUTHOR(S): Iyengar, Sujatha; Shah, Keerti V.; Kotloff, Karen L.; Ghim, Shin-Je; Viscidi, Raphael P.
CORPORATE SOURCE: Department Molecular Microbiology and Immunology, Johns Hopkins School Hygiene and Public Health, Baltimore, MD, 21205-2179, USA
SOURCE: Clinical and Diagnostic Laboratory Immunology (1996), 3(6), 733-739
CODEN: CDIMEN; ISSN: 1071-412X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The human papillomavirus type 16 (HPV-16) L1 capsid protein is the major component of the HPV virion. We prepd. L1 protein of HPV-16 in a **cell-free** system. The L1 gene was cloned in an expression plasmid and transcribed and translated in vitro in a rabbit reticulocyte lysate. The expressed protein had the mol. mass (55 kDa) expected for the L1 protein, and it assembled into virus-like particles that closely resembled papillomavirus virions. The protein retained conformational epitopes, as evidenced by its reactivity with monoclonal antibodies which recognize only intact viral particles. In radioimmunopptn. assays with sera from college women grouped by their genital tract HPV DNA status, high reactivity was found in 68% of HPV-16 DNA-pos. women, in 23% of women with other HPVs, and in 19% of HPV-neg. women. In comparison, none of the sera of children were reactive. The results of the radioimmunopptn. assays showed a significant correlation with results obtained with the same sera in an ELISA with virus-like particles produced in baculovirus (chi-square test for linear trend, P = 0.0023). Although the amts. of L1 protein obtained are small, the ability to produce virus-like particles by in vitro translation may be useful in the study of virus assembly, virus binding, and the immunol. response to HPV infection.

FILE 'BIOSIS' ENTERED AT 12:04:46 ON 31 OCT 2002
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=> crystalization
L1 139 CRYSTALIZATION

=> protein and L1
L2 15 PROTEIN AND L1

=> virus and L2
L3 1 VIRUS AND L2

=> difficulty and L2
L4 0 DIFFICULTY AND L2

=> D L3 IBIB TI SO AU ABS

L3 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1977:166932 BIOSIS
DOCUMENT NUMBER: BA63:61796
TITLE: FOLDING OF DNA MOLECULES IN CHROMATIN.
AUTHOR(S): DAMASCHUN G
SOURCE: ACTA BIOL MED GER, (1975 (RECD 1976)) 34 (11-12), NO PAGE.
CODEN: ABMGAJ. ISSN: 0001-5318.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
TI FOLDING OF DNA MOLECULES IN CHROMATIN.
SO ACTA BIOL MED GER, (1975 (RECD 1976)) 34 (11-12), NO PAGE.
CODEN: ABMGAJ. ISSN: 0001-5318.
AU DAMASCHUN G
AB A structural model for the folding of DNA in chromatin based on the X-ray diffraction patterns of deoxyribonucleoproteins (DNP) is presented. The DNA is oriented in the direction of DNP fibers and does not exhibit a superhelical structure. In the .nu.-bodies the DNA is folded 7 times on the envelope of a cylinder 10 nm in diameter. The height of the DNA hairpins is 9 nm-10 nm. The spacing between the refolded DNA segments is 3.6 nm. This supramolecular folding **crystalization** of the DNA is a general principle of organization and, through different types of morphological growth of the folding crystals, leads to the chromatin, to .psi.-DNA, to DNA monocrystals and to DNA packing in some phage heads.

binding of NCp7 to ifs nucleic acid targets. The mechanism of these activities is still debated but several clues are in favor of an intermediate aggregation of nucleic acids by NCp7. To check and characterize the nucleic acid aggregating properties of NCp7, we investigated the interaction of NCp7 with the model RNA homopolymer, polyA, by quasielastic tight scattering and optical density measurements. The ordered growth of monodisperse large particles independently of the nucleic acid size and the almost complete covering of polyA by NCp7 strongly suggested an ordered aggregation mechanism. The aggregate kinetics of growth in the optimum protein concentration range (greater than or equal to 2 mu M) were governed by a so-called Ostwald ripening mechanism limited by transfer of NCp7-covered polyA complexes from small to large aggregates. The aggregation process was strongly dependent on both Na+ at and Mg2+ concentrations, the optimum concentrations being in the physiological range. Similar conclusions held true when polyA was replaced by 16S+23S ribosomal RNA, suggesting that the NCp7 aggregating properties were only poorly dependent on the nucleic acid sequence and structure. Finally, as in the NCp7 annealing activities, the basic regions of NCp7, but not the zinc fingers, were found critical in nucleic acid aggregation. Taken together, our data indicate that NCp7 is a highly efficient nucleic acid aggregating agent and strengthen the hypothesis that aggregation may constitute a transient step in various NCp7 functions. (C) 1997 John Wiley & Sons, Inc.

L9 ANSWER 18 OF 44 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998087828 MEDLINE
DOCUMENT NUMBER: 98087828 PubMed ID: 9426448
TITLE: Identification of a specific interaction between the coronavirus mouse hepatitis **virus** A59 **nucleocapsid protein** and packaging signal.
AUTHOR: Molenkamp R; Spaan W J
CORPORATE SOURCE: Department of Virology, Institute of Medical Microbiology, Leiden University, The Netherlands.
SOURCE: VIROLOGY, (1997 Dec 8) 239 (1) 78-86.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980206
Last Updated on STN: 19980206
Entered Medline: 19980129

AB The coronavirus mouse hepatitis virus (MHV) is an enveloped positive stranded RNA virus. In infected cells MHV produces a 3' coterminal nested set of subgenomic messenger RNAs. Only the genomic RNA, however, is encapsidated by the nucleocapsid protein and incorporated in infectious MHV virions. It is believed that an RNA packaging signal (Ps), present only in the genomic RNA, is responsible for this selectivity. Earlier studies mapped this signal to a 69-nt stem-loop structure positioned in the 3' end of ORF1b. The selective encapsidation mechanism probably initiates by specific interaction of the packaging signal with the nucleocapsid protein. In this study we demonstrate the in **vitro** interaction of the MHV-A59 nucleocapsid protein with the packaging signal of MHV

using gel retardation and UV cross-linking assays. This interaction was observed not only with the nucleocapsid protein from infected cells but also with that from purified virions and from cells expressing a recombinant nucleocapsid protein. The specificity of the interaction was demonstrated by competition experiments with nonlabeled Ps containing RNAs, **tRNA**, and total cytoplasmic RNA. The results indicated that no virus specific modification of the N-protein or the presence of other viral proteins are required for this in **vitro** intervention. The assays described in this report provide us with a powerful tool for studying encapsidation (initiation) in more detail.

L9 ANSWER 19 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 97:44832 SCISEARCH
THE GENUINE ARTICLE: WA713
TITLE: NCp7 activates HIV-1(Lai) RNA dimerization by converting a transient loop-loop complex into a stable dimer
AUTHOR: Muriaux D; DeRocquigny H; Roques B P; Paoletti J (Reprint)
CORPORATE SOURCE: INST GUSTAVE ROUSSY, CNRS, URA 147, UNITE BIOCHIM, 39 RUE CAMILLE DESMOULINS, F-94805 VILLEJUIF, FRANCE (Reprint); INST GUSTAVE ROUSSY, CNRS, URA 147, UNITE BIOCHIM, F-94805 VILLEJUIF, FRANCE; UNIV PARIS 05, CNRS, URA D1500, INSERM, U266, DEPT PHARACOCHIM MOL & STRUCT, F-75006 PARIS, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 DEC 1996) Vol. 271, No. 52, pp. 33686-33692.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 55

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Nucleocapsid **protein** 7 (NCp7), the human immunodeficiency **virus** type 1 (HTV-1) **nucleocapsid protein**, was shown to strongly potentiate the dimerization of the retroviral genomic RNA. This process involves the inter action of two retroviral RNA monomer subunits near their 5'-ends. A region located upstream from the splice donor site was recently identified as being responsible for the formation of dimeric HIV-1 RNA. This region appeared to be confined within a stem-loop structure, with an autocomplementary sequence in the loop. In an in **vitro** study of spontaneous dimer formation, we reported that the 77-402 RNA transcript forms two distinct dimers differing in their thermostability: D37 and D55. We identified D37 as a ''kissing'' complex structure, formed via a loop-loop interaction between the two monomers, and D55 as a double stranded structure involving all nucleotides of the stem loop via canonical base pairing. In this report, we have characterized the role of NCp7 in the HIV-1(Lai) RNA dimerization process by using in **vitro** dimerization assays with RNA transcripts of different lengths and dimer thermal dissociation. Our results show that the nucleocapsid protein NCp7 activates RNA dimerization very likely through interaction with the kissing complex and converts it into a stable

dimer. Furthermore, this NCp7-prometed conversion only occurs if the 240-280 stem loop structure is present in HIV-1(Lai) RNA molecules and contains the autocomplementary G(257)CGCGC(262) sequence. This study suggests that, under physiological conditions, an NCp7-mediated RNA conformational change is involved in the maturation of the HIV-1 RNA dimer.

L9 ANSWER 20 OF 44 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 96323089 MEDLINE
DOCUMENT NUMBER: 96323089 PubMed ID: 8709195
TITLE: The zinc finger of **nucleocapsid protein** of Friend murine leukemia **virus** is critical for proviral DNA synthesis in vivo.
AUTHOR: Yu Q; Darlix J L
CORPORATE SOURCE: LaboRetro, Institut National de la Sante et de la Recherche Medicale U412, Ecole Normale Superieure de Lyon, France.
SOURCE: JOURNAL OF VIROLOGY, (1996 Sep) 70 (9) 5791-8. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19980206
Entered Medline: 19960910

AB **Nucleocapsid protein** NCp10 of murine leukemia **virus** (MuLV) is encoded by the 3' domain of gag and contains a zinc finger of the form Cys-X2-Cys-X4-His-X4-Cys flanked by basic amino acids. In the course of virus assembly, NCp10 is necessary for core formation, and the zinc finger flanked by the basic residues is required for the packaging of the genomic RNA dimer. In **vitro**, NCp10 exhibits strong nucleic acid binding and annealing activities that appear to be critical for virus infectivity since NCp10 promotes dimerization of the viral RNA containing the E/DLS packaging-dimerization signal and annealing of replication primer **tRNA**(Pro) to the initiation site of reverse transcription (PBS). Recent in **vitro** studies have suggested that NCp10 may also play a role in proviral DNA synthesis. To investigate the function of NCp10 in proviral DNA synthesis in vivo, we developed a simple and convenient genetic packaging system consisting of two DNA constructs expressing the packaging components gag-pol and env of Friend MuLV and a Moloney MuLV-based lacZ vector with either the MuLV E+ or a rat VL30 E packaging signal. This system allowed us to examine the consequences of a set of mutations in NCp10 on a single round of recombinant virus replication. Most mutations in the N- or C-terminal domain of NCp10 do not significantly alter infectivity, while those in the zinc finger drastically impair infectivity. Analysis of the viral RNA content in virions showed that all mutations in the zinc finger decrease but do not abolish packaging of the recombinant genome. Interestingly enough, mutation of Y-28 to S (mutation Y28S) in the zinc finger results in RNA packaging at a level similar to that observed upon deletion of three prolines and three arginines in the C-terminal domain of NCp10 (mutant delta PR3). However, mutant Y28S is noninfectious while mutant delta PR3 is only threefold less

infectious than the wild-type virus, which prompted us to examine the role of NCp10 protein in proviral DNA synthesis in vivo using these nucleocapsid mutants. PCR amplification was used to analyze viral DNA synthesized in newly infected cells, and results indicate that the Y28S zinc finger mutation impairs reverse transcription, thus suggesting that the nucleocapsid protein zinc finger plays a key role in proviral DNA synthesis in vivo.

L9 ANSWER 21 OF 44 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 96357022 MEDLINE
DOCUMENT NUMBER: 96357022 PubMed ID: 8764006
TITLE: Human immunodeficiency **virus** Type 1 **nucleocapsid protein** (NCp7) directs specific initiation of minus-strand DNA synthesis primed by human **tRNA**(Lys3) in **vitro** : studies of viral RNA molecules mutated in regions that flank the primer binding site.
AUTHOR: Li X; Quan Y; Arts E J; Li Z; Preston B D; de Rocquigny H; Roques B P; Darlix J L; Kleiman L; Parniak M A; Wainberg M A
CORPORATE SOURCE: McGill University AIDS Center, Lady Davis Institute-Jewish General Hospital, Montreal, Quebec, Canada.
SOURCE: JOURNAL OF VIROLOGY, (1996 Aug) 70 (8) 4996-5004. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961107

AB Retroviral reverse transcription starts near the 5' end of unspliced viral RNA at a sequence called the primer binding site (PBS), where the **tRNA** primer anneals to the RNA template for initiation of DNA synthesis. We have investigated the roles of NCp7 in annealing of primer **tRNA**(Lys3) to the PBS and in reverse transcriptase (RT) activity, using a cell-free reverse transcription reaction mixture consisting of various 5' viral RNA templates, natural primer **tRNA**(Lys3) or synthetic primer, human immunodeficiency **virus** type I (HIV-1) **nucleocapsid protein** (NCp7), and HIV-1 RT. In the presence of **tRNA**(Lys3), NCp7 was found to stimulate synthesis of minus-strand strong-stop DNA [(-)ssDNA], consistent with previous reports. However, specific DNA synthesis was observed only at a NCp7/RNA ratio similar to that predicted to be present in virions. Moreover, at these concentrations, NCp7 inhibited the synthesis of nonspecific reverse-transcribed DNA products, which are initiated because of self-priming by RNA templates. In contrast to results obtained with **tRNA**(Lys3) as primer, NCp7 inhibited the synthesis of (-)ssDNA products primed by an 18-nucleotide (nt) ribonucleotide (rPR), complementary to the PBS, even though rPR can initiate synthesis of such material in the absence of preannealing with NCp7. Primer placement band shift assays showed that NCp7 was necessary for efficient formation of the **tRNA**-RNA complex. In contrast, NCp7 was found to prevent formation of the rPR-RNA complex. Since NCp7 appears to exert opposite effects (annealing

versus dissociation) on **tRNA**(Lys3) and rPR substrates, the non-PBS binding regions of the **tRNA**(Lys3) molecule may play a role in the annealing of **tRNA** to the template. We also investigated the roles of an A-rich loop upstream of the PBS, a 7-nt region immediately downstream of the PBS, and a 54-nt deletion further downstream of the PBS in interactions with **tRNA** (Lys3). We found that deletions in the 54-nt region that may prevent formation of the U5-leader stem prevented **tRNA**(Lys3) placement and priming, while deletions in the A-rich loop or the 7-nt sequence had relatively minor effects in this regard.

L9 ANSWER 22 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 96:73111 SCISEARCH
THE GENUINE ARTICLE: TP526
TITLE: MUTATIONS OF BASIC-AMINO-ACIDS OF NCP7 OF HUMAN-IMMUNODEFICIENCY-VIRUS TYPE-1 AFFECT RNA-BINDING IN-**VITRO**
AUTHOR: SCHMALZBAUER E; STRACK B; DANNULL J; GUEHMANN S; MOELLING K (Reprint)
CORPORATE SOURCE: UNIV ZURICH, INST MED VIROL, GLORIASTR 30, CH-8028 ZURICH, SWITZERLAND (Reprint); UNIV ZURICH, INST MED VIROL, CH-8028 ZURICH, SWITZERLAND
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: JOURNAL OF VIROLOGY, (FEB 1996) Vol. 70, No. 2, pp. 771-777.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **nucleocapsid** (NC) **protein** of human immunodeficiency **virus** type 1 is required for packaging of viral RNA and for virion assembly. It contains two clusters of basic amino acids, consisting of five and four amino acid residues, flanking the first of its two zinc fingers, These amino acid residues have been mutagenized to neutral ones individually, as well as in various combinations, by site-directed mutagenesis. Wild-type NCp7 and the mutant proteins were expressed as recombinant proteins in Escherichia coli, with six histidines as tags at their amino termini in order to allow efficient purification. The purified proteins were analyzed for RNA binding in **vitro** with human immunodeficiency virus type 1 5' leader RNA transcribed in **vitro**. Assays comprised Northwestern blots at various salt concentrations and filter binding tests which allowed determination of the dissociation constants of the various mutants, The results indicated that mutations of the amino acid R-7 and of R-32 and K-33 were more critical for RNA binding than other mutations. Mutation of the other amino acid residues reduced the binding affinity in proportion to the number of mutations, Mutation of seven of the nine basic amino acid residues reduced the binding of RNA by 50- to 90-fold.

L9 ANSWER 23 OF 44 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96215651 MEDLINE
DOCUMENT NUMBER: 96215651 PubMed ID: 8645104
TITLE: RNA binding properties of core protein of the flavivirus Kunjin.

AUTHOR: Khromykh A A; Westaway E G
CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Brisbane, Australia.
SOURCE: ARCHIVES OF VIROLOGY, (1996) 141 (3-4) 685-99. Journal code: 7506870. ISSN: 0304-8608.
PUB. COUNTRY: Austria
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960726
Last Updated on STN: 19980206
Entered Medline: 19960715

AB Kunjin virus (KUN) C is a typical flavivirus core protein which is truncated in vivo to a mature form of 105 residues enriched in lysine and arginine. In order to study the possible association of KUN C with RNA in **vitro**, we prepared several recombinant C proteins with specific deletions, each fused at the amino-terminus to glutathione-S-transferase (GST) and expressed in E. coli. They were reacted with KUN RNA probes transcribed in **vitro** from cDNA representing the 5' untranslated region (5' UTR, 93 to 96 nucleotides), the 3' UTR (624 nucleotides), and the 5' UTR plus most of the C coding region (5' core, 440 nucleotides). Fusion protein C107 (incorporating mature C) bound strongly to all KUN RNA probes with apparent specificity, being completely resistant to inhibition by 800 mM NaCl, and to competition by a large excess of **tRNA** . In reactions with labelled KUN RNA probes putative binding sites were identified in the isolated amino-terminal (32 residues) and carboxy-terminal (26 residues) basic amino acid domains; this binding was strongly competed by unlabelled KUN UTR probes but weakly or not at all by **tRNA**. These small domains probably acted co-operatively in binding of mature C to KUN RNA probes. The KUN RNA-core protein binding reactions are similar to those reported with other **viral** coat or **capsid proteins** and **viral** RNAs.

L9 ANSWER 24 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 96:894836 SCISEARCH
THE GENUINE ARTICLE: VV421
TITLE: A specific RNA structural motif mediates high affinity binding by the HIV-1 nucleocapsid protein (NCp7)
AUTHOR: Allen P (Reprint); Collins B; Brown D; Hostomsky Z; Gold L
CORPORATE SOURCE: UNIV COLORADO, DEPT MOL CELLULAR & DEV BIOL, CAMPUS BOX 347, BOULDER, CO 80309 (Reprint); AGOURON PHARMACUET INC, SAN DIEGO, CA 92121
COUNTRY OF AUTHOR: USA
SOURCE: VIROLOGY, (15 NOV 1996) Vol. 225, No. 2, pp. 306-315
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0042-6822.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 45
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Current research indicates that the **nucleocapsid protein** (NCp7) of human immunodeficiency **virus** type 1 (HIV-1) interacts with a variety of RNA substrates during the progression of the viral life cycle. The RNA features specifically recognized by the protein, however, have yet to be identified. SELEX was used to generate a set of RNAs whose affinities for nucleocapsid were on the order of 2 x 10(-9) M. Comparative analysis revealed that each RNA contains a highly conserved fourteen nucleotide sequence-block. Computer modeling and structure probing experiments indicate that the RNA ligands use the consensus sequence to fold into hairpins with an identical asymmetric bulge. The presence of the nucleocapsid protein protects the asymmetric bulge from ribonuclease attack, suggesting that it is the key element in protein recognition. A search for similar structural motifs within the HIV genome reveals several potential interaction sites for the nucleocapsid protein. (C) 1996 Academic Press, Inc.

L9 ANSWER 25 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 11
ACCESSION NUMBER: 1996:228892 BIOSIS
DOCUMENT NUMBER: PREV199698785021
TITLE: Mutations at the capsid-nucleocapsid cleavage site of gag polyprotein of Moloney murine leukemia virus abolish virus infectivity.
AUTHOR(S): Housset, Valerie; Darlix, Jean-Luc (1)
CORPORATE SOURCE: (1) LaboRetro, Unite Virol. Hum., INSERM-ENS U412, Ec. Norm. Super. Lyon, 46 allee d'Italie, 69634 Lyon Cedex France
SOURCE: Comptes Rendus de l'Academie des Sciences Serie III Sciences de la Vie, (1996) Vol. 319, No. 2, pp. 81-89.
ISSN: 0764-4469.
DOCUMENT TYPE: Article
LANGUAGE: English; French
SUMMARY LANGUAGE: English; French
AB Capsid protein CAp30 and nucleocapsid protein NCp10 of Moloney murine leukemia virus (MoMuLV) are the 2 major proteic components of the virion core and are generated by processing of the gag polyprotein precursor, Pr65-gag, by the viral protease. In the virion core, several hundred NCp10 molecules are bound to the genomic RNA dimer forming the **nucleocapsid** structure. In the course of **virus** assembly, NC **protein**, as the mature NCp10 and/or as the gag precursor, appears to direct genomic RNA packaging. In **vitro**, NCp10 has nucleic acid binding and annealing activities and promotes viral RNA dimerization and the annealing of replication primer **tRNA**-Pro to the primer binding site (PBS) which is necessary for the initiation of reverse transcription. To investigate whether maturation of NCp10 is required for virus formation, we substituted charged residues for the hydrophobic amino acids at the capsid-nucleocapsid protein cleavage site in order to prevent maturation of NCp10. Here we report that these mutations abolished maturation of capsid protein CAp30 and NCp 10 by the viral protease in **vitro**. When these mutations were introduced into an infectious MoMuL V molecular clone, Pr65-gag precursor was synthesized in transfected cells but virion production was strongly diminished and mutant viruses were not infectious. These results suggest that maturation of NCp10 is required for optimal virion release and production of infectious

virus.

L9 ANSWER 26 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 12
ACCESSION NUMBER: 95102944 EMBASE
DOCUMENT NUMBER: 1995102944
TITLE: Synthesis and conformational studies of a cyclic analog of the proximal zinc finger of HIV-1 NCP7 for antibody generation.
AUTHOR: Dong C.Z.; Jullian N.; Yang Y.S.; De Rocquigny H.; Fournie-Zaluski M.C.; Roques B.P.
CORPORATE SOURCE: Dept. de Pharmacochim.Molec./Struct., CNRS URA D 150, Faculte de Pharmacie, 4 Avenue de l'Observatoire,75270 Paris Cedex 06, France
SOURCE: Journal of the American Chemical Society, (1995) 117/10 (2726-2731).
ISSN: 0002-7863 CODEN: JACSAT
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus** type 1 (HIV-1) contains two zinc finger domains (CCHC boxes), which have been shown to be very important in the viral replication cycle. However, these domains are not involved directly in either in **vitro** RNA dimerization or **tRNA**(Lys,3) annealing. For a more detailed understanding of the role of the zinc fingers in the different functions of NCp7, antibodies directed against these domains would be very useful. For this purpose, a cyclic peptide analog of the proximal zinc finger (13-30)NCp7 has been synthesized in solid phase using a strategy of combined Fmoc and Boc chemistry. On the basis of the 3D structural data of NCp7, the Asn17 and Ala30 have been changed to Glu17 and Lys30 and a cyclization carried out between their side chains. The structures of the cyclic and native peptides complexed with Co2+ and Zn2+ were studied by visible and 2D 1H NMR spectroscopy, respectively. The nuclear Overhauser effects obtained were applied as constraints to determine the solution structures using DIANA software followed by AMBER energy refinement. The results show that the cyclic peptide retains the highly folded structure of the native peptide and exhibits an enhanced affinity for metallic ions. These are favorable parameters for the generation of antibodies against the zinc fingers in NCp7.

L9 ANSWER 27 OF 44 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 95130539 MEDLINE
DOCUMENT NUMBER: 95130539 PubMed ID: 7829498
TITLE: Binding of the HIV-1 nucleocapsid protein to the primer **tRNA**(3Lys), in **vitro**, is essentially not specific.
AUTHOR: Mely Y; de Rocquigny H; Sorinas-Jimeno M; Keith G; Roques B P; Marquet R; Gerard D
CORPORATE SOURCE: Laboratoire de Biophysique, URA 491 du CNRS, Universite Louis Pasteur de Strasbourg I, Faculte de Pharmacie, Illkirch, France.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jan 27) 270 (4) 1650-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950307
Last Updated on STN: 19990129
Entered Medline: 19950222

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus**, type 1, is a key component in the viral life cycle. Since, the first common step of all reported NCp7 activities corresponds to a nucleic acid-binding step, the NCp7 binding parameters to the natural primer **tRNA**(3Lys) were investigated. Using NCp7 intrinsic fluorescence, we found that (i) in 0.1 M NaCl, NCp7 bound noncooperatively to **tRNA**(3Lys) with a Kobs = 3.2 x 10(6) M-1 association constant and a n = 6 binding site size, (ii) four ionic interactions were formed in the NCp7.**tRNA**(3Lys) complex, and (iii) nonelectrostatic factors provided about 60% of the binding energy. These binding parameters were not significantly altered when the natural **tRNA**(3Lys) was replaced by either an in **vitro** synthetic **tRNA**(3Lys) transcript, the heterologous yeast **tRNA**(Phe) or the structurally unrelated 5 S RNA from Escherichia coli. Moreover, the environment of the intrinsic fluorescent reporters (Trp37 and Trp61) was similar in the various complexes. Finally, experiments performed at low protein concentration provide no evidence of high affinity binding sites. Taken together, our data strongly suggested an essentially nonspecific binding of NCp7 to **tRNA**(3Lys) and thus did not seem to support a direct role of NCp7, per se, in the selection of **tRNA**(3Lys) from the pool of cellular **tRNAs**.

L9 ANSWER 28 OF 44 MEDLINE DUPLICATE 14

ACCESSION NUMBER: 95001879 MEDLINE
DOCUMENT NUMBER: 95001879 PubMed ID: 7918387
TITLE: 1H NMR structure and biological studies of the His23-->Cys mutant nucleocapsid protein of HIV-1 indicate that the conformation of the first zinc finger is critical for virus infectivity.
AUTHOR: Demene H; Dong C Z; Ottmann M; Rouyez M C; Jullian N; Morellet N; Mely Y; Darlix J L; Fournie-Zaluski M C; Saragosti S; +
CORPORATE SOURCE: Departement de Pharmacochimie Moleculaire et Structurale, U266 INSERM-URA D1500 CNRS, Faculte de Pharmacie, Universite Rene Descartes, Paris, France.
SOURCE: BIOCHEMISTRY, (1994 Oct 4) 33 (39) 11707-16.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 20000303
Entered Medline: 19941108

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus** type 1 (HIV-1), which has key functions in the virus life cycle, possesses two zinc fingers of the CX2CX4HX4C type characterized by three successive loops containing a tetrahedrally coordinated zinc atom. The replacement of any cysteine by a serine in either finger has been shown to result in the production of noninfectious viruses, probably by impairing the biological functions of NCp7. In order to more precisely elucidate the structural role of the zinc finger motif, His23 was replaced by Cys in the proximal finger of the peptide (13-64)NCp7 which retains NCp7 activities in **vitro**. The peptide Cys23(13-64)NCp7 was synthesized by solid phase and studied by 2D 1H NMR and molecular modeling. The His to Cys modification causes important structural modifications of the N-terminal zinc finger which impair the spatial proximity of the two zinc fingers as shown by the disappearance of several interresidue NOEs. The side chains of Val13, Lys14, Phe16, Thr24, Ala25, Trp37, Gln45, and Met46, which are thought to be involved in nucleic acid recognition, are no longer found clustered in the Cys23(13-64)NCp7 mutant as they are in the wild-type NCp7 structure. In **vitro**, Cys23(13-64)NCp7 is unable to tightly interact with the viral RNA or replication primer **tRNA** (Lys,3). The Cys23(NCp7) mutation was introduced into an infectious HIV-1 molecular clone, and virions produced upon DNA transfection into cells were analyzed for their viral protein and RNA compositions as well as for their infectivity. Results show that, while the Cys23(NCp7) mutation does not impair virion production, viruses contain a low amount of degraded viral RNA and are not infectious. These findings suggest that a bona fide conformation of the HIV-1 NCp7 is critical for the packaging of viral RNA, its stability in virions, and virus infectivity.

L9 ANSWER 29 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 94:235001 SCISEARCH
THE GENUINE ARTICLE: NF928
TITLE: SPECIFIC BINDING OF HIV-1 NUCLEOCAPSID PROTEIN TO PSI-RNA IN-**VITRO** REQUIRES N-TERMINAL ZINC-FINGER AND FLANKING BASIC-AMINO-ACID RESIDUES
AUTHOR: DANNULL J; SUROVOY A; JUNG G; MOELLING K (Reprint)
CORPORATE SOURCE: UNIV ZURICH, INST MED VIROL, CH-8028 ZURICH, SWITZERLAND (Reprint); MAX PLANCK INST MOLEC GENET, SCHUSTER ABT, D-14195 BERLIN, GERMANY; UNIV TUBINGEN, INST ORGAN CHEM, D-72076 TUBINGEN, GERMANY
COUNTRY OF AUTHOR: SWITZERLAND; GERMANY
SOURCE: EMBO JOURNAL, (01 APR 1994) Vol. 13, No. 7, pp. 1525-1533.
ISSN: 0261-4189.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **nucleocapsid** (NC) **protein** of human immunodeficiency **virus** HIV-1 (NCp7) isresponsible for packaging the viral RNA by recognizing a packaging site (PSI)on the viral RNA genome. NCp7 is a molecule of 55 amino acids containing two zinc fingers, with only the first one being highly conserved among retroviruses. The first zinc finger is flanked by two basic amino acid clusters. Here we demonstrate that chemically synthesized

NCp7 specifically binds to viral RNA containing the PSI using competitive filter binding assays. Deletion of the PSIfrom the RNA abrogates this effect. The 35 N-terminal amino acids of NCp7, comprising the first zinc finger, are sufficient for specific RNA binding. Chemically synthesized mutants of the first zinc finger demonstrate that the amino acid residues C-C-C/H-C/H are required for specific RNA binding and zinc coordination. Amino acid residues F16 and T24, but not K20, E21 and G22, located within this zinc finger, are essential for specific RNA binding as well. The second zinc finger cannot replace the first one. Furthermore, mutations in the basic amino acid residues flanking the first zinc finger demonstrate that R3, 7,10, 29 and 32 but not K11, 14, 33 and 34 are also essential for specific binding. Specific binding to viral RNA is also observed with recombinant NCp15 andPr55Gag. The results demonstrate for the first time specific interaction of aretroviral NC protein with its PSI RNA in **vitro**.

L9 ANSWER 30 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 94:145398 SCISEARCH
THE GENUINE ARTICLE: MX771
TITLE: TRANSACTIVATION OF THE MINUS-STRAND DNA TRANSFER BY NUCLEOCAPSID PROTEIN DURING REVERSE TRANSCRIPTION OF THE RETROVIRAL GENOME
AUTHOR: ALLAIN B; LAPADATTAPOLSKY M; BERLIOZ C; DARLIX J L (Reprint)
CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, 46 ALLEE ITALIE, F-69364 LYON, FRANCE (Reprint); ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, F-69364 LYON, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: EMBO JOURNAL, (15 FEB 1994) Vol. 13, No. 4, pp. 973-981.
ISSN: 0261-4189.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two DNA strand transfers are required during reverse transcription of the RNA genome of retroviruses to complete provirus synthesis. To understand more about the first strand transfer reaction, that of the minus-strand DNA from the 5' to the 3' end of the retroviral genome, we devised an in **vitro** system mimicking the Moloney murine leukemia virus reverse transcription process. Two RNAs corresponding to the 5' and 3' regions of the genome were used to perform reverse transcription assays. The role of the nucleocapsid protein NCp10, which is tightly bound to the genome in the virus, was investigated in this system as well as the requirement of the 5' and 3' terminal repeats (R sequences) and the poly(A) tail. The results show that NCp10 drastically enhances the strand transfer reaction and that interactions between reverse transcriptase, **nucleocapsid protein** and **viral** RNA may be important. Both R sequences are required for an efficient and accurate DNA strand transfer and the poly(A) tail facilitates this reaction. Furthermore, it is probable that both intra- and intermolecular DNA strand transfers occur when the 5' and 3' ends of the genome are present on the same molecule.

L9 ANSWER 31 OF 44 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 93281729 MEDLINE
DOCUMENT NUMBER: 93281729 PubMed ID: 8506369
TITLE: Identification of a binding site for the human immunodeficiency **virus** type 1 **nucleocapsid protein.**
AUTHOR: Sakaguchi K; Zambrano N; Baldwin E T; Shapiro B A; Erickson J W; Omichinski J G; Clore G M; Gronenborn A M; Appella E
CORPORATE SOURCE: Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20892.
CONTRACT NUMBER: N01-CO-74102 (NCI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Jun 1) 90 (11) 5219-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930716
Last Updated on STN: 19980206
Entered Medline: 19930707

AB The nucleocapsid (NC) protein NCp7 of human immunodeficiency virus type 1 (HIV-1) is important for encapsidation of the virus genome, RNA dimerization, and primer **tRNA** annealing in **vitro**. Here we present evidence from gel mobility-shift experiments indicating that NCp7 binds specifically to an RNA sequence. Two complexes were identified in native gels. The more slowly migrating complex contained two RNA molecules and one peptide, while the more rapidly migrating one is composed of one RNA and one peptide. Further, mutational analysis of the RNA shows that the predicted stem and loop structure of stem-loop 1 plays a critical role. Our results show that NCp7 binds to a unique RNA structure within the psi region; in addition, this structure is necessary for RNA dimerization. We propose that NCp7 binds to the RNA via a direct interaction of one zinc-binding motif to stem-loop 1 followed by binding of the other zinc-binding motif to stem-loop 1, stem-loop 2, or the linker region of the second RNA molecule, forming a bridge between the two RNAs.

L9 ANSWER 32 OF 44 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 93233217 MEDLINE
DOCUMENT NUMBER: 93233217 PubMed ID: 8474159
TITLE: Basic amino acids flanking the zinc finger of Moloney murine leukemia **virus nucleocapsid protein** NCp10 are critical for **virus** infectivity.
AUTHOR: Housset V; De Rocquigny H; Roques B P; Darlix J L
CORPORATE SOURCE: LaboRetro Institut National de la Sante et de la Recherche Medicale, Ecole Normale Superieure de Lyon, France.
SOURCE: JOURNAL OF VIROLOGY, (1993 May) 67 (5) 2537-45.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199305
ENTRY DATE: Entered STN: 19930604
Last Updated on STN: 19970203
Entered Medline: 19930517

AB Nucleocapsid (NC) protein NCp10 of Moloney murine leukemia virus is encoded by the 3' domain of gag and contains a zinc finger surrounded by basic amino acids. During virion assembly, NC protein is necessary for core formation and the NC zinc finger is required for the packaging of the genomic RNA dimer. In **vitro** NCp10 has RNA-binding and -annealing activities critical for virus infectivity, since NCp10 promotes dimerization of viral RNA containing the Psi packaging element and annealing of replication primer **tRNA**(Pro) to the initiation site of reverse transcription (primer-binding site). To investigate the role of the basic amino acids flanking the NCp10 zinc finger, neutral residues were substituted for the basic amino acids and the effects of these mutations in vivo on virus assembly and infectivity and in **vitro** on the RNA-annealing activity of NCp10 were analyzed. Here we report that the substitution of 1 or 2 neutral amino acids for the basic residues did not impair the production of mature virions but that infectivity was either moderately or strongly attenuated. When more than 2 basic residues were replaced by neutral amino acids, viruses were poorly infectious because of a severe defect in genomic RNA dimer packaging and initiation of reverse transcription. In **vitro** NCp10-derived peptides with similar mutations were chemically synthesized and were found to be either fully or partially active or completely inactive. These data indicate that the basic residues flanking the zinc finger of NCp10 are required for the production of infectious Moloney murine leukemia virus virions.

L9 ANSWER 33 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 93:171068 SCISEARCH
THE GENUINE ARTICLE: KR189
TITLE: INTERACTIONS BETWEEN HIV-1 **NUCLEOCAPSID PROTEIN** AND **VIRAL**-DNA MAY HAVE IMPORTANT FUNCTIONS IN THE VIRAL LIFE-CYCLE
AUTHOR: LAPADATTAPOLSKY M; DEROCQUIGNY H; VANGENT D; ROQUES B; PLASTERK R; DARLIX J L (Reprint)
CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, LABORETRO INSERM, 46 ALLEE DITALIE, F-69364 LYON, FRANCE; UNIV PARIS 05, UNITE PHARMACOCHIM MOLEC & STRUCT, CNRS, URA D1500, INSERM, U266, F-75270 PARIS 06, FRANCE; NETHERLANDS CANC INST, DIV MOLEC BIOL, 1066 CX AMSTERDAM, NETHERLANDS
COUNTRY OF AUTHOR: FRANCE; NETHERLANDS
SOURCE: NUCLEIC ACIDS RESEARCH, (25 FEB 1993) Vol. 21, No. 4, pp. 831-839.
ISSN: 0305-1048.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In the virion core of retroviruses, the genomic RNA is tightly associated with nucleocapsid (NC) protein molecules, forming the nucleocapsid structure. NC protein, a highly basic protein with two

zinc fingers, is indispensable for RNA dimerization, encapsidation and the initiation of reverse transcription in avian, murine and human retroviruses. Here we show that NC protein of HIV-1 (NCp7) and NCp7 mutants bind to DNA fragments representing proviral DNA sequences, forming stable complexes. NCp7 and NCp7 mutants form high molecular weight complexes with large DNA fragments and show cooperativity in binding to the DNAs. It appears that the conserved basic residues, and not the zinc fingers, are important for complex formation. In addition, NCp7 and several NCp7 mutants protect DNAs from nuclease digestion while the DNA ends appear to be poorly protected. NCp7 has been found to bind to strong stop cDNA, the initial product of reverse transcription, and to promote the annealing of this cDNA to HIV-1 RNA corresponding to the 3' end of the genome. In addition, NCp7 slightly stimulates an in **vitro** IN cleavage assay. These results indicate that the interactions between NCp7 and proviral DNA may be important during provirus synthesis and/or prior to integration.

L9 ANSWER 34 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 93:592908 SCISEARCH
THE GENUINE ARTICLE: LY812
TITLE: TRANSACTIVATION OF THE 5' TO 3' **VIRAL**-DNA STRAND TRANSFER BY **NUCLEOCAPSID** **PROTEIN** DURING REVERSE TRANSCRIPTION OF HIV-1 RNA
AUTHOR: DARLIX J L (Reprint); VINCENT A; GABUS C; DEROCQUIGNY H; ROQUES B
CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, 46 ALLEE ITALIE, F-69364 LYON 07, FRANCE (Reprint); UNIV PARIS 05, FAC PHARM, INSERM, U266, F-75270 PARIS, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: COMPTES RENDUS DE L ACADEMIE DES SCIENCES SERIE III-SCIENCES DE LA VIE-LIFE SCIENCES, (AUG 1993) Vol. 316, No. 8, pp. 763-771.
ISSN: 0764-4469.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Two DNA strand transfer reactions take place during reverse transcription of the retroviral genome. The first transfer, that of the minus-strand strong stop DNA from the 5' end of the viral RNA to the 3' end, has been studied in **vitro** with two RNAs mimicking the 5' and 3' regions of the HIV1 genome and with nucleocapsid protein, NCp7, and reverse transcriptase. The results show that NCp7 strongly activates the 5' to 3' DNA strand transfer during reverse transcription while a basic peptide resembling NCp7 is inactive. Activation of the first transfer by several NCp7 derived peptides and the influence of the terminal redundancies (R) present at the 5' and 3' ends of HIV1 RNA were also examined The first transfer is optimal in the presence of intact NCp7 and necessitates R on both the 5' and 3' RNAs. Sequencing of full length viral DNA products reveals approximately 40 % misincorporations at the first nucleotide beyond the transfer point. If such base misincorporations occur during proviral DNA synthesis with possible homologous recombinations it may well contribute to the high level

of genetic variability of HIV.

L9 ANSWER 35 OF 44 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 92335320 MEDLINE
DOCUMENT NUMBER: 92335320 PubMed ID: 1631144
TITLE: Viral RNA annealing activities of human immunodeficiency **virus** type 1 **nucleocapsid protein** require only **peptide** domains outside the zinc fingers.
AUTHOR: De Rocquigny H; Gabus C; Vincent A; Fournie-Zaluski M C; Roques B; Darlix J L
CORPORATE SOURCE: Departement de Chimie Organique, U266 Institut National de la Sante et de la Recherche Medicale Unite Associee 498, Universite Rene Descartes, Paris, France.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Jul 15) 89 (14) 6472-6.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920904
Last Updated on STN: 19980206
Entered Medline: 19920818

AB The nucleocapsid (NC) of human immunodeficiency virus type 1 consists of a large number of NC protein molecules, probably wrapping the dimeric RNA genome within the virion inner core. NC protein is a gag-encoded product that contains two zinc fingers flanked by basic residues. In human immunodeficiency virus type 1 virions, NCp15 is ultimately processed into NCp7 and p6 proteins. During virion assembly the retroviral NC protein is necessary for core formation and genomic RNA encapsidation, which are essential for virus infectivity. In **vitro** NCp15 activates viral RNA dimerization, a process most probably linked in vivo to genomic RNA packaging, and replication primer **tRNA**(Lys,3) annealing to the initiation site of reverse transcription. To characterize the domains of human immunodeficiency virus type 1 NC protein necessary for its various functions, the 72-amino acid NCp7 and several derived peptides were synthesized in a pure form. We show here that synthetic NCp7 with or without the two zinc fingers has the RNA annealing activities of NCp15. Further deletions of the N-terminal 12 and C-terminal 8 amino acids, leading to a 27-residue peptide lacking the finger domains, have little or no effect on NC protein activity in **vitro**. However deletion of short sequences containing basic residues flanking the first finger leads to a complete loss of NC protein activity. It is proposed that the basic residues and the zinc fingers cooperate to select and package the genomic RNA in vivo. Inhibition of the viral RNA binding and annealing activities associated with the basic residues flanking the first zinc finger of NC protein could therefore be used as a model for the design of antiviral agents.

L9 ANSWER 36 OF 44 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 93077033 MEDLINE
DOCUMENT NUMBER: 93077033 PubMed ID: 1280240

TITLE: Recombinant HIV-1 nucleocapsid protein p15 produced as a fusion protein with glutathione S-transferase in Escherichia coli mediates dimerization and enhances reverse transcription of retroviral RNA.
AUTHOR: Weiss S; Konig B; Morikawa Y; Jones I
CORPORATE SOURCE: Boehringer Mannheim GmbH, Research Center Penzberg, Germany.
SOURCE: GENE, (1992 Nov 16) 121 (2) 203-12.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19990129
Entered Medline: 19921230

AB Human immunodeficiency **virus** 1 (HIV-1) **nucleocapsid protein** p15 was produced as a fusion protein with glutathione S-transferase (GST) in Escherichia coli. Rapid purification of GST::p15 in an active form by one-step glutathione-agarose chromatography was accomplished in the presence of an antioxidant. Recombinant p15 fused to GST was shown to stimulate the dimerization of viral RNA. HIV-1 reverse transcriptase-catalyzed in **vitro** synthesis of minus-strand cDNA from synthetic human **tRNA**(Lys3UUU) and natural bovine **tRNA**(Lys3SUU) primer molecules was enhanced by GST::p15. GST produced in E.coli revealed no effect with respect to RNA dimerization and cDNA synthesis, demonstrating that both activities reside in the p15 portion of the fusion protein.

L9 ANSWER 37 OF 44 MEDLINE DUPLICATE 19
ACCESSION NUMBER: 91305094 MEDLINE
DOCUMENT NUMBER: 91305094 PubMed ID: 1906602
TITLE: **Viral** RNA annealing activities of the **nucleocapsid protein** of Moloney murine leukemia **virus** are zinc independent.
AUTHOR: Prats A C; Housset V; de Billy G; Cornille F; Prats H; Roques B; Darlix J L
CORPORATE SOURCE: Centre de Recherche de Biochimie et Genetique Cellulaires du CNRS, Toulouse, France.
SOURCE: NUCLEIC ACIDS RESEARCH, (1991 Jul 11) 19 (13) 3533-41.
Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910908
Last Updated on STN: 20000303
Entered Medline: 19910820

AB The zinc fingers of retroviral gag nucleocapsid proteins (NC) are required for the specific packaging of the dimeric RNA genome into virions. In **vitro**, NC proteins activate both dimerization of viral RNA and annealing of the replication primer **tRNA** onto viral RNA, two reactions necessary for the production of infectious virions. In this study the role of the zinc finger of

Moloney murine leukemia virus (MoMuLV) NCp10 in RNA binding and annealing activities was investigated through modification or replacement of residues involved in zinc coordination. These alterations did not affect the ability of NCp10 to bind RNA and promote RNA annealing in **vitro**, despite a complete loss of zinc affinity. However mutation of two conserved lysine residues adjacent to the finger motif reduced both RNA binding and annealing activities of NCp10. These findings suggest that the complexed NC zinc finger is not directly involved in RNA-protein interactions but more probably in a zinc dependent conformation of NC protein modulating viral protein-protein interactions, essential to the process of viral RNA selection and virion assembly. Then the NC zinc finger may cooperate to select the viral RNA genome to be packaged into virions.

L9 ANSWER 38 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1990:3604 BIOSIS
DOCUMENT NUMBER: BA89:3604
TITLE: HIV-1 REVERSE TRANSCRIPTASE SPECIFICALLY INTERACTS WITH THE ANTICODON DOMAIN OF ITS COGNATE PRIMER **TRANSFER RNA**.
AUTHOR(S): BARAT C; LULLIEN V; SCHATZ O; KEITH G; NUGEYRE M T; GRUENINGER-LEITCH F; BARRE-SINOUSSI F; LEGRICE S F J; DARLIX J L
CORPORATE SOURCE: LABO-RETRO CENT. DE RECHERCHE DE BIOCHIMIE ET GENETIQUE CELLULAIRES DU CNRS, 118 ROUTE DE NARBONNE, F-31062 TOULOUSE CEDEX, FRANCE.
SOURCE: EMBO (EUR MOL BIOL ORGAN) J, (1989) 8 (11), 3279-3286.
CODEN: EMJODG. ISSN: 0261-4189.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The virion cores of the replication type 1 human immunodeficiency virus (HIV-1), a retrovirus, contain an RNA genome associated with nucleocapsid (NC) and erverse transcriptase (RT p66/p51) molecules. In **vitro** reconstructions of these complexes with purified components show that NC is required for efficient annealing of the primer tRNALys,3. In the absence of NC, HIV-1 RT is unable to retrotranscribe the viral RNA template from the **tRNA** primer. We demonstrate that the HIV-1 RT p66/p51 specifically binds to its cognate primer tRNALys,3 even in the presence of a 100-fold molar excess of other **tRNAs**. Cross-linking analysis of this interaction locates the contact site to a region within the heavily modified anti-codon domain of tRNALys,3.

L9 ANSWER 39 OF 44 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 87106837 MEDLINE
DOCUMENT NUMBER: 87106837 PubMed ID: 2433189
TITLE: Translation of homologous and heterologous messenger RNAs in a yeast cell-free system.
AUTHOR: Hussain I; Leibowitz M J
CONTRACT NUMBER: GM-32413 (NIGMS)
SOURCE: GENE, (1986) 46 (1) 13-23.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 198703
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19970203
Entered Medline: 19870319

AB A stable mRNA-dependent cell-free translation system from Saccharomyces cerevisiae, prepared by a modification of the method of Hofbauer et al. [Eur. J. Biochem. 122 (1982) 199-203] was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addition of polyamines and yeast **tRNA**. The m transcript of the M segment of double-stranded RNA, synthesized in **vitro** using the killer virus-associated RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunologically identified using antitoxin antibody. Sindbis **virus capsid protein** and rabbit globin were also translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin and anisomycin. Analogues of the 5'-terminal caps present on most eukaryotic mRNA molecules inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

L9 ANSWER 40 OF 44 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 86068014 MEDLINE
DOCUMENT NUMBER: 86068014 PubMed ID: 2999784
TITLE: Direct mapping of adeno-associated **virus capsid proteins** B and C: a possible ACG initiation codon.
AUTHOR: Becerra S P; Rose J A; Hardy M; Baroudy B M; Anderson C W
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1985 Dec) 82 (23) 7919-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198601
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860106

AB The three major **capsid proteins** of adeno-associated **virus** type 2 (AAV2) virions are designated A, B, and C and have molecular sizes of 90, 72, and 60 kDa, respectively. These proteins are related, and genetic studies have shown they are encoded by a long open reading frame located in the right half of the genome. The coding capacity distal to the first ATG in this reading frame is only 503 amino acids (i.e., a protein about the size of protein C), but an open frame sequence devoid of ATG codons extends upstream for an additional 184 codons. Although the amino terminus of the C capsid protein is blocked, partial amino acid sequence analyses of peptides from C have confirmed that it is encoded within the portion of the reading frame distal to the first ATG at nucleotide (nt) location 2810. The amino terminus of the B capsid protein is not blocked, and its sequence begins with alanine. The triplet encoding this alanine lies 64 codons upstream from the initiation site for protein C and is immediately preceded by the threonine codon, ACG, at nt 2615. This

ACG codon lies in the most favorable sequence context for protein synthesis initiation. All three AAV2 capsid proteins are labeled in **vitro** with formyl[35S]methionyl-tRNAf, indicating that synthesis of each protein is initiated independently. Our data suggest that the nt 2615 ACG codon directs the methionyl-**tRNA**-dependent initiation of the AAV2 B capsid protein. Proteins B and C may be synthesized from the same mRNA species and their relative abundance could be determined by the efficiencies of their respective initiation codons.

L9 ANSWER 41 OF 44 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 79007648 MEDLINE
DOCUMENT NUMBER: 79007648 PubMed ID: 211268
TITLE: Polyoma virus has three late mRNA's: one for each virion protein.
AUTHOR: Siddell S G; Smith A E
SOURCE: JOURNAL OF VIROLOGY, (1978 Aug) 27 (2) 427-31. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197811
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19970203
Entered Medline: 19781129

AB Polyoma virus mRNA, isolated from the cytoplasm of 3T6 cells late after infection and purified by hybridization to HpaII fragment 3 of polyoma virus DNA, was separated on 50% formamide-containing sucrose density gradients, and the fractionated RNA was recovered and translated in **vitro**. Analysis of the cell-free products showed that the minor virion protein VP3 was synthesized from an mRNA sedimenting at approximately 18S betweeen the 19S VP2 mRN and the 16S VP1 mRNA. Other experiments showed that the VP2 and VP3 can be labeled with formyl methionine from initiator **tRNA**. We conclude that there are three late polyoma virus mRNA's, each directing the synthesis of only one **viral capsid protein**.

L9 ANSWER 42 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 79030132 EMBASE
DOCUMENT NUMBER: 1979030132
TITLE: Characterization of the mRNA's for the polyoma **virus capsid proteins** VP1, VP2, and VP3.
AUTHOR: Hunter T.; Gibson W.
CORPORATE SOURCE: Tum. Virol. Lab., Salk Inst., San Diego, Calif. 92112, United States
SOURCE: Journal of Virology, (1978) 28/1 (240-253). CODEN: JOVIAM
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
029 Clinical Biochemistry
LANGUAGE: English

AB Polyadenylated cytoplasmic RNA from polyoma virus-infected cells can be translated in the wheat germ system to yield all three polyoma **virus capsid proteins**, VP1, VP2, and

VP3. The translation products of RNA selected from total cytoplasmic RNA of infected cells by hybridization to polyoma virus DNA showed a high degree of enrichment for VP1, VP2, and VP3. The identity of the in **vitro** products with authentic viron proteins was established in two ways. First, tryptic peptide maps of the in **vitro** products were found to be essentially identical to those of their in vivo counterparts. Second, the mobilities of the in **vitro** products on two-dimensional gels were the same as those of viral proteins labeled in vivo. VP1, VP2, and VP3 were all labeled with [35S]formylmethionine when they were synthesized in the presence of [35S]formylmethionyl-**tRNA**(f)(met). We determined the sizes of the polyadenylated mRNA's for VP1, VP2, and VP3 by fractionation on gels. The sizes of the major mRNA species for the capsid proteins are as follows: VP2, 8.5 x 105 daltons; VP3, 7.4 x 105 daltons; and VP1, 4.6 x 105 daltons. We conclude that all three **viral capsid proteins** are synthesized independently in **vitro**, that all three **viral capsid proteins** are virally coded, and that each of the capsid proteins has a discrete mRNA.

L9 ANSWER 43 OF 44 MEDLINE DUPLICATE 23
ACCESSION NUMBER: 77013481 MEDLINE
DOCUMENT NUMBER: 77013481 PubMed ID: 1067601
TITLE: Initiation of translation directed by 42S and 26S RNAs from Semliki Forest virus in **vitro**.
AUTHOR: Glanville N; Ranki M; Morser J; Kaariainen L; Smith A E
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1976 Sep) 73 (9) 3059-63.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197612
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19761201

AB The proteins synthesized in **vitro** in response to 42S and 26S RNAs from Semliki Forest virus were labeled with formyl-[35S]methionine from initiator **tRNA**. One **protein** which comigrated with **viral capsid protein** was labeled under the direction of 26S RNA, and only one labeled peptide was detected after digestion with trypsin. Further digestion with pronase gave rise to the dipeptide fMet-AsN. Several labeled polypeptides were found in the 42S RNA directed product and these had molecular weights of up to 150,000. However, tryptic digestion of the product yielded only one formylmethionyl-labeled peptide, which had a different mobility from that directed by the 26S RNA. Further digestion with pronase gave a single dipeptide, fMet-Ala. This indicates that nonstructural proteins as large as 150,000 daltons are probably synthesized from one initiation site on the 42S RNA. Translation starting from the internal initiation site on the 42S RNA, which is equivalent to that on the 26S RNA, could not be detected under the conditions used. Internal initiation sites which are similarly inactive have also been detected in other viral RNAs (e.g., brome mosaic virus, tobacco

mosaic virus, and polyoma 19S RNA) and this suggests that, although eukaryotic mRNAs can contain more than one initiation site for protein synthesis, only the site nearer the 5' terminus is active in **vitro**.

L9 ANSWER 44 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 76175635 EMBASE
DOCUMENT NUMBER: 1976175635
TITLE: Initiation sites for translation of Sindbis virus 42S and 26S messenger RNAs.
AUTHOR: Cancedda R.; Villa Komaroff L.; Lodish H.F.; Schlesinger M.
CORPORATE SOURCE: II Fac. Med., Univ. Naples, Italy
SOURCE: Cell, (1975) 6/2 (215-222).
CODEN: CELLB5
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
022 Human Genetics
LANGUAGE: English
AB Sindbis virus 26S RNA is the principal species of virus specific RNA found in the infected cell; it is derived from a one third segment of virion 42S RNA. When translated in cell free extracts from mouse ascites cells or rabbit reticulocytes, 26S RNA directed the synthesis primarily of the 33,000 dalton **virus capsid protein**, and the **protein** products were in the form of free peptides rather than peptidyl **tRNA**. In contrast, the polypeptides synthesized in either extract in response to Sindbis virus 42S RNA were heterogeneous, ranging in molecular weight from 33,000 to 190,000, and were largely in the form of peptidyl **tRNA**. The number of independent initiation sites on the 26S and 42S RNAs was determined by analyzing a tryptic digest of reaction products labeled with yeast N formyl 35S methionyl **tRNA**(F)(MET). The 26S RNA appeared to contain a single initiation site, and this site could also be found in varying amounts in different preparations of 42S RNA. However, a second initiation site, distinct from that of 26S RNA was the major site in 42S virion RNA. These results suggest that 42S virion RNA contains two potential sites for initiation of protein synthesis. Only one of these may be active, however, and it is postulated that the second site functions primarily, if not exclusively, in the subgenomic 26S RNA species. In this regard, Sindbis virus 42S RNA may represent a novel form of a eucaryotic messenger RNA.

=> fil hom
FILE 'HOME' ENTERED AT 12:52:52 ON 08 OCT 2002

(FILE 'HCAPLUS' ENTERED AT 12:24:29 ON 08 OCT 2002)
L1 29954 SEA FILE=HCAPLUS ABB=ON PLU=ON TRNA OR (T OR TRANSFER?) (W)(RNA OR RIBONUCLEIC OR RIBO NUCLEIC)
L5 4555 SEA FILE=HCAPLUS ABB=ON PLU=ON (PEPTIDE OR PROTEIN OR POLYPROTEIN OR POLYPEPTIDE)(5A)((HCV OR HEPATIT? C OR FLAVIVIR? OR VIRUS OR VIRAL)(5A)(NUCLEOCAPSID? OR CAPSID? OR PSEUDONUCLEOCAPSID?))
L6 54 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND L5
L7 25 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND VITRO

L7 ANSWER 1 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:414964 HCAPLUS
DOCUMENT NUMBER: 137:180722
TITLE: Functional interactions of **nucleocapsid protein** of feline immunodeficiency **virus** and cellular prion **protein** with the viral RNA
AUTHOR(S): Moscardini, Mila; Pistello, Mauro; Bendinelli, M.; Ficheux, Damien; Miller, Jennifer T.; Gabus, Caroline; Le Grice, Stuart F. J.; Surewicz, Witold K.; Darlix, Jean-Luc
CORPORATE SOURCE: Department of Biomedicine, University of Pisa, Pisa, I-56127, Italy
SOURCE: Journal of Molecular Biology (2002), 318(1), 149-159
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB All lentiviruses and oncoretroviruses examd. so far encode a major nucleic-acid binding protein (nucleocapsid or NC* protein), approx. 2500 mols. of which coat the dimeric RNA genome. Studies on HIV-1 and MoMuLV using in **vitro** model systems and in vivo have shown that NC protein is required to chaperone viral RNA dimerization and packaging during virus assembly, and proviral DNA synthesis by reverse transcriptase (RT) during infection. The human cellular prion protein (PrP), thought to be the major component of the agent causing transmissible spongiform encephalopathies (TSE), was recently found to possess a strong affinity for nucleic acids and to exhibit chaperone properties very similar to HIV-1 NC protein in the HIV-1 context in **vitro**. Tight binding of PrP to nucleic acids is proposed to participate directly in the prion disease process. To extend our understanding of lentiviruses and of the unexpected nucleic acid chaperone properties of the human prion protein, we set up an in **vitro** system to investigate replication of the feline immunodeficiency virus (FIV), which is functionally and phylogenetically distant from HIV-1. The results show that in the FIV model system, NC protein chaperones viral RNA dimerization, primer tRNALys,3 annealing to the genomic primer-binding site (PBS) and minus strand DNA synthesis by the homologous FIV RT. FIV NC protein is able to trigger specific viral DNA synthesis by inhibiting self-priming of reverse transcription. The human prion protein was found to mimic the properties of FIV NC with respect to primer **tRNA** annealing to the viral RNA and chaperoning minus strand DNA synthesis.
REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 2 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:357237 HCAPLUS
DOCUMENT NUMBER: 137:89946
TITLE: Conformational Changes Accompanying Self-Assembly of the Hepatitis C Virus Core Protein
AUTHOR(S): Kunkel, Meghan; Watowich, Stanley J.
CORPORATE SOURCE: Department of Human Biological Chemistry & Genetics and the Sealy Center for Strucutral Biology, University of Texas Medical Branch, Galveston, TX, 77555, USA
SOURCE: Virology (2002), 294(2), 239-245
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Elsevier Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Although a no. of recent studies have suggested that the function of the hepatitis C virus (HCV) core protein may be both to package the viral genome and to modulate host cellular processes, little is known of the structure of the core protein necessary to accomplish these functions. Using in **vitro** assembled particles that mimic essential features of native HCV nucleocapsids, we report the earliest structural information of the **HCV** core **protein** and its **nucleocapsid**. The core protein is proteinase-resistant when assembled into nucleocapsid-like particles or complexed with nucleic acid in **vitro**. In contrast, the highly basic amino terminus of the free core protein is sensitive to proteolytic digestion. The hydrophobic carboxyl-terminal region of the core protein stabilizes the structure of the free core protein but is not required to stabilize core protein assembled into nucleocapsid-like particles or complexed to nucleic acid. Significantly, the carboxyl-terminal region is sufficient, but not necessary, to fold the core protein into a stable structure. These data are consistent with a model of a partially flexible HCV core protein that undergoes extensive conformational changes upon binding to nucleic acid and assembling into nucleocapsid particles. In addn., the susceptibility of nucleocapsid particles to RNase digestion suggests that RNA-core interactions may stabilize HCV nucleocapsids.
REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 3 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:352529 HCAPLUS
DOCUMENT NUMBER: 133:262220
TITLE: In **vitro** studies on **tRNA** annealing and reverse transcription with mutant HIV-1 RNA templates
AUTHOR(S): Beerens, Nancy; Berkhout, Ben
CORPORATE SOURCE: Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Amsterdam, 1100 DE, Neth.
SOURCE: Journal of Biological Chemistry (2000), 275(20), 15474-15481
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular

Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The human immunodeficiency virus type 1 (HIV-1) RNA genome encodes a semistable stem-loop structure, the U5-PBS hairpin, which occludes part of the **tRNA** primer binding site (PBS). In previous studies, we demonstrated that mutations that alter the stability of the U5-PBS hairpin inhibit virus replication. A reverse transcription defect was measured in assays with the virion-extd. RNA-**tRNA** complexes. We now extend these studies with in **vitro** synthesized wild-type and mutant RNA templates that were tested in primer annealing and reverse transcription assays. The effect of annealing temp. and the presence of the **viral nucleocapsid protein** on reverse transcription was analyzed for the templates with a stabilized or destabilized U5-PBS hairpin, and in reactions initiated by **tRNA** or DNA primers. The results of this in **vitro** assay are consistent with the in vivo findings, in that both **tRNA** annealing and initiation of reverse transcription are sensitive to stable template RNA structure. Reverse transcription initiated by a DNA primer is less hindered by secondary structure in the RNA template than **tRNA** primed reactions. The inhibitory effect of template structure on **tRNA**-primed reverse transcription is more pronounced in this in **vitro** assay compared with the in vivo material, indicating that the heat-annealed RNA-**tRNA** complex differs from the virion-extd. viral RNA-**tRNA** complex.
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 4 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:275903 HCAPLUS
DOCUMENT NUMBER: 133:40377
TITLE: Nucleic acid-dependent cross-linking of the **nucleocapsid protein** of Sindbis **virus**
AUTHOR(S): Tellinghuisen, Timothy L.; Kuhn, Richard J.
CORPORATE SOURCE: Department of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA
SOURCE: Journal of Virology (2000), 74(9), 4302-4309
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The assembly of the alphavirus nucleocapsid core is a multistep event requiring the assocn. of the nucleocapsid protein with nucleic acid and the subsequent oligomerization of capsid proteins into an assembled core particle. Although the mechanism of assembly has been investigated extensively both in vivo and in **vitro**, no intermediates in the core assembly pathway have been identified. Through the use of both truncated and mutant Sindbis **virus nucleocapsid proteins** and a variety of crosslinking reagents, a possible nucleic acid-protein assembly intermediate has been detected. The cross-linked species, a covalent dimer, has been detected only in the presence of nucleic acid and with capsid proteins capable of binding nucleic acid. Optimum nucleic acid-dependent crosslinking was seen at a

protein-to-nucleic-acid ratio identical to that required for max. binding of the capsid protein to nucleic acid. Identical results were obsd. when crosslinking in **vitro** assembled core particles of both Sindbis and Ross River viruses. Purified cross-linked dimers of truncated proteins and of mutant proteins that failed to assemble were found to incorporate into assembled core particles when present as minor components in assembly reactions, suggesting that the crosslinking traps an authentic intermediate in nucleocapsid core assembly. Endoproteinase Lys-C mapping of the position of the cross-link indicated that lysine 250 of one capsid protein was cross-linked to lysine 250 of an adjacent capsid protein. Examn. of the position of the cross-link in relation to the existing model of the nucleocapsid core suggests that the cross-linked species is a cross-capsomere contact between a pentamer and hexamer at the quasi-threefold axis or is a cross-capsomere contact between hexamers at the threefold axis of the icosahedral core particle and suggests several possible assembly models involving a nucleic acid-bound dimer of capsid protein as an early step in the assembly pathway.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 5 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:77926 HCAPLUS
DOCUMENT NUMBER: 132:217923
TITLE: In **vitro** studies of human **tRNA** (lys,3) primer annealing by human immunodeficiency **virus** type 1 **nucleocapsid protein**
AUTHOR(S): Chan, Chunkong Barden
CORPORATE SOURCE: Univ. of Minnesota, Minneapolis, MN, USA
SOURCE: (1999) 152 pp. Avail.: UMI, Order No. DA9934942 From: Diss. Abstr. Int., B 1999, 60(6), 2668
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L7 ANSWER 6 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:598851 HCAPLUS
DOCUMENT NUMBER: 131:320081
TITLE: Characterization of the block in replication of nucleocapsid protein zinc finger mutants from Moloney murine leukemia virus
AUTHOR(S): Gorelick, Robert J.; Fu, William; Gagliardi, Tracy D.; Bosche, William J.; Rein, Alan; Henderson, Louis E.; Arthur, Larry O.
CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National Cancer Institute Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201, USA
SOURCE: Journal of Virology (1999), 73(10), 8185-8195 CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mutagenesis studies have shown that retroviral nucleocapsid (NC) protein Zn2+ fingers (-Cys-X2-Cys-X4-His-X4-Cys- [CCHC]) perform

multiple functions in the virus life cycle. Moloney murine leukemia virus mutants His 34.fwdarw.Cys (CCCC) and Cys 39.fwdarw.His (CCHH) were able to package their genomes normally but were replication defective. Thermal dissocn. expts. showed that the CCHH mutant was not defective in genomic RNA dimer structure. Primer **tRNA** placement on the viral genome and the ability of the **tRNA** to function in reverse transcription initiation in **vitro** also appear normal. Some "full-length" DNA copies of the viral genome were synthesized in mutant virus-infected cells. The CCCC and CCHH mutants produced these DNA copies at greatly reduced levels. Circle junction fragments, amplified from 2-long-terminal-repeat viral DNA (vDNA) by PCR, were cloned and characterized. VDNA isolated from cells infected with mutant virions had a wide variety of abnormalities at the site at which the 2 ends of the linear precursor had been ligated to form the circle (i.e., the junction between the 5' end of U3 and the 3' end of U5). In some mols., bases were missing from regions corresponding to the U3 and U5 linear vDNA termini; in others, the viral sequences extended either beyond the U5 sequences into the primer-binding site and 5' leader or beyond the U3 sequences into the polypurine tract into the env coding region. Still other mols. contained nonviral sequences between the linear vDNA termini. Such defective genomes would certainly be unsuitable substrates for integration. Thus, strict conservation of the CCHC structure in NC is required for infection events prior to and possibly including integration.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:263474 HCAPLUS
DOCUMENT NUMBER: 131:98386
TITLE: The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of **tRNA** on the primer binding site
AUTHOR(S): Feng, Ya-Xiong; Campbell, Stepfen; Harvin, Demetria; Ehresmann, Bernard; Ehresmann, Chantal; Rein, Alan
CORPORATE SOURCE: Retroviral Genetics Section, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702, USA
SOURCE: Journal of Virology (1999), 73(5), 4251-4256
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The formation of an infectious retrovirus particle requires several RNA-RNA interaction events. In particular, the genomic RNA mols. form a dimeric structure, and a cellular **tRNA** mol. is annealed to an 18-base complementary region (the primer binding site, or PBS) on the genomic RNA, where it will serve as primer for reverse transcription. **TRNAs** normally possess a highly stable secondary and tertiary structure; it seems unlikely that annealing of a **tRNA** mol. to the PBS, which involves unwinding of this structure, could occur efficiently at physiol.

temps. without the assistance of a cofactor. Many prior studies have shown that the **viral nucleocapsid** (NC) **protein** can act as a nucleic acid chaperone (i.e., facilitate annealing events between nucleic acids), and the assays used to demonstrate this activity include its ability to catalyze dimerization of transcripts representing retroviral genomes and the annealing of **tRNA** to the PBS in **vitro**. However, mature NC is not required for these events in vivo, since protease-deficient viral mutants, in which NC is not cleaved from the parental Gag polyprotein, are known to contain dimeric RNAs with **tRNA** annealed to the PBS. In the present expts., we have tested recombinant human immunodeficiency virus type 1 Gag polyprotein for nucleic acid chaperone activity. The protein was pos. by all of our assays, including the ability to stimulate dimerization and to anneal **tRNA** to the PBS in **vitro**. In quant. expts., its activity was approx. equiv. on a molar basis to that of NC. Based on these results, we suggest that the Gag polyprotein (presumably by its NC domain) catalyzes the annealing of **tRNA** to the PBS during (or before) retrovirus assembly in vivo.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:172329 HCAPLUS
DOCUMENT NUMBER: 130:307463
TITLE: Role of post-transcriptional modifications of primer tRNALys,3 in the fidelity and efficacy of plus strand DNA transfer during HIV-1 reverse transcription
AUTHOR(S): Auxilien, Sylvie; Keith, Gerard; Le Grice, Stuart F. J.; Darlix, Jean-Luc
CORPORATE SOURCE: LaboRetro ENS, INSERM U412, Lyon, 69364, Fr.
SOURCE: Journal of Biological Chemistry (1999), 274(7), 4412-4420
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB During HIV reverse transcription, (+) strand DNA synthesis is primed by an RNase H-resistant sequence, the polypurine tract, and continues as far as a 18-nt double-stranded RNA region corresponding to the 3' end of tRNALys,3 hybridized to the viral primer binding site (PBS). Before (+) strand DNA transfer, reverse transcriptase (RT) needs to unwind the double-stranded **tRNA**-PBS RNA in order to reverse-transcribe the 3' end of primer tRNALys,3. Since the detailed mechanism of (+) strand DNA transfer remains incompletely understood, we developed an in **vitro** system to closely examine this mechanism, composed of HIV 5' RNA, natural modified tRNALys,3, synthetic unmodified tRNALys,3 or oligonucleotides (RNA or DNA) complementary to the PBS, as well as the **viral proteins** RT and **nucleocapsid protein** (NCp7). Prior to (+) strand DNA transfer, RT stalls at the double-stranded **tRNA**-PBS RNA complex and is able to reverse-transcribe modified nucleosides of natural tRNALys,3. Modified nucleoside m1A-58 of natural tRNALys,3 is only partially

effective as a stop signal, as RT can transcribe as far as the hyper-modified adenosine (ms2t6A-37) in the anticodon loop. M1A-58 is almost always transcribed into A, whereas other modified nucleosides are transcribed correctly, except for m7G-46, which is sometimes transcribed into T. In contrast, synthetic tRNALys,3, an RNA PBS primer, and a DNA PBS primer are completely reverse-transcribed. In the presence of an acceptor template, (+) strand DNA transfer is efficient only with templates contg. natural tRNALys,3 or the RNA PBS primer. Sequence anal. of transfer products revealed frequent errors at the transfer site with synthetic tRNALys,3, not obsd. with natural tRNALys,3. Thus, modified nucleoside m1A-58, present in all retroviral **tRNA** primers, appears to be important for both efficacy and fidelity of (+) strand DNA transfer. We show that other factors such as the nature of the (-) PBS of the acceptor template and the RNase H activity of RT also influence the efficacy of (+) strand DNA transfer.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:167604 HCAPLUS
DOCUMENT NUMBER: 131:55342
TITLE: Binding Properties of the Human Immunodeficiency **Virus** Type 1 **Nucleocapsid Protein** p7 to a Model RNA: Elucidation of the Structural Determinants for Function
AUTHOR(S): Urbaneja, Maria A.; Kane, Bradley P.; Johnson, Donald G.; Gorelick, Robert J.; Henderson, Louis E.; Casas-Finet, Jose R.
CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201, USA
SOURCE: Journal of Molecular Biology (1999), 287(1), 59-75
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB HIV-1 nucleocapsid protein (NCp7) is a double zinc-fingered protein that has been traditionally implicated in viral RNA recognition and packaging, in addn. to its tight assocn. with genomic RNA and **tRNA** primer within the virion nucleocapsid. The availability of large quantities of viral or recombinant wild-type NCp7 and mutant p7 has made possible the assignment of the different roles that structural motifs within the protein play during RNA binding. At low ionic strength binding to the homopolymeric fluorescent RNA, poly(.epsilon.A), is electrostatically driven and four sodium ions are displaced. Arg7 in the flanking N-terminal region, Lys20 and Lys26 in the first zinc finger and one pos. charged residue (attributed to Lys41) in the second zinc finger are involved in electrostatic contacts with RNA. The p7 zinc fingers do not function independently but concomitantly. The first zinc finger (both isolated or in the context of the full-length protein) has a more prominent electrostatic interaction than the second one. The second zinc finger dominates the non-electrostatic stabilization of

the binding to RNA due to stacking of its Trp residue with nucleic acid bases. Mutations in the highly conserved retroviral Zn-coordinating residues (CCHC) to steroid hormone receptor (CCCC) or transcription factor (CCHH) metal cluster types do not affect RNA binding. In spite of the limited impact in RNA binding affinity in **vitro** or RNA packaging in vivo that such mutations or structural alterations impart, they impair or abolish virus infectivity. It is likely that such an effect stems from the involvement of NCp7 in crucial steps of the virus life cycle other than RNA binding. (c) 1999 Academic Press.

REFERENCE COUNT: 80 THERE ARE 80 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 10 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:578140 HCAPLUS
DOCUMENT NUMBER: 129:286701
TITLE: The yeast Ty3 retrotransposon contains a 5'-3' bipartite primer-binding site and encodes nucleocapsid protein NCp9 functionally homologous to HIV-1 NCp7
AUTHOR(S): Gabus, Caroline; Ficheux, Damien; Rau, Michael; Keith, Gerard; Sandmeyer, Suzanne; Darlix, Jean-Luc
CORPORATE SOURCE: LaboRetro, Unite de Virologie Humaine, INSERM (#412), Ecole Normale Superieure de Lyon, Lyon, 69364, Fr.
SOURCE: EMBO Journal (1998), 17(16), 4873-4880
CODEN: EMJODG; ISSN: 0261-4189
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Retroviruses, including HIV-1 and the distantly related yeast retroelement Ty3, all encode a nucleoprotein required for virion structure and replication. During an in **vitro** comparison of HIV-1 and Ty3 nucleoprotein function in RNA dimerization and cDNA synthesis, we discovered a bipartite primer-binding site (PBS) for Ty3 composed of sequences located at opposite ends of the genome. Ty3 cDNA synthesis requires the 3' PBS for primer tRNA1 Met annealing to the genomic RNA, and the 5' PBS, in cis or in trans, as the reverse transcription start site. Ty3 RNA alone is unable to dimerize, but formation of dimeric tRNAiMet bound to the PBS was found to direct dimerization of Ty3 RNA-tRNAiMet. Interestingly, HIV-1 nucleocapsid protein NCp7 and Ty3 NCp9 were interchangeable using HIV-1 and Ty3 RNA template-primer systems. Our findings impact on the understanding of non-canonical reverse transcription as well as on the use of Ty3 systems to screen for anti-NCp7 drugs.

L7 ANSWER 11 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:802603 HCAPLUS
DOCUMENT NUMBER: 128:111499
TITLE: The nucleocapsid protein specifically anneals tRNALys-3 onto a noncomplementary primer binding site within the HIV-1 RNA genome in **vitro**
AUTHOR(S): Chan, Barden; Musier-Forsyth, Karin
CORPORATE SOURCE: Dep. Chem., Univ. Minnesota, Minneapolis, MN, 55455, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1997), 94(25), 13530-13535
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB HIV type 1 (HIV-1) specifically uses host cell tRNALys-3 as a primer for reverse transcription. The 3' 18 nucleotides of this **tRNA** are complementary to a region on the HIV RNA genome known as the primer binding site (PBS). HIV-1 has a strong preference for maintaining a lysine-specific PBS in vivo, and viral genomes with mutated PBS sequences quickly revert to be complementary to tRNALys-3. To investigate the mechanism for the obsd. PBS reversion events in **vitro**, we examd. the capability of the nucleocapsid protein (NC) to anneal various **tRNA** primer sequences onto either complementary or noncomplementary PBSs. We show that NC can anneal different full-length **tRNAs** onto viral RNA transcripts derived from the HIV-1 MAL or HXB2 isolates, provided that the PBS is complementary to the **tRNA** used. In contrast, NC promotes specific annealing of only tRNALys-3 onto an RNA template (HXB2) whose PBS sequence has been mutated to be complementary to the 3' 18 nt of human tRNAPro. Moreover, HIV-1 reverse transcriptase extends this binary complex from the proline-specific PBS. The formation of the noncomplementary binary complex does not occur when a chimeric tRNALys/Pro contg. proline-specific D and anticodon domains is used as the primer. Thus, elements outside the acceptor-T.psi.C domains of tRNALys-3 play an important role in preferential primer use in **vitro**. Our results support the hypothesis that mutant PBS reversion is a result of tRNALys-3 annealing onto and extension from a PBS that specifies an alternate host cell **tRNA**.

L7 ANSWER 12 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:789372 HCAPLUS
DOCUMENT NUMBER: 128:85609

TITLE: Identification of a specific interaction between the coronavirus mouse hepatitis **virus** A59 **nucleocapsid protein** and packaging signal

AUTHOR(S): Molenkamp, Richard; Spaan, Willy J. M.
CORPORATE SOURCE: Department of Virology, Institute of Medical Microbiology, Leiden University, AZL-L4-Q, Leiden, 2300 RC, Neth.

SOURCE: Virology (1997), 239(1), 78-86
CODEN: VIRLAX; ISSN: 0042-6822
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The coronavirus mouse hepatitis virus (MHV) is an enveloped pos. stranded RNA virus. In infected cells MHV produces a 3' coterminal nested set of subgenomic mRNAs. Only the genomic RNA, however, is encapsidated by the nucleocapsid protein and incorporated in infectious MHV virions. It is believed that an RNA packaging signal (Ps), present only in the genomic RNA, is responsible for this selectivity. Earlier studies mapped this signal to a 69-nt stem-loop structure positioned in the 3' end of ORF1b. The selective encapsidation mechanism probably initiates by specific

interaction of the packaging signal with the nucleocapsid protein. In this study we demonstrate the in **vitro** interaction of the MHV-A59 nucleocapsid protein with the packaging signal of MHV using gel retardation and UV crosslinking assays. This interaction was obsd. not only with the nucleocapsid protein from infected cells but also with that from purified virions and from cells expressing a recombinant nucleocapsid protein. The specificity of the interaction was demonstrated by competition expts. with nonlabeled Ps contg. RNAs, **tRNA**, and total cytoplasmic RNA. The results indicated that no virus specific modification of the N protein or the presence of other viral proteins are required for this in **vitro** interaction. The assays described in this report provide us with a powerful tool for studying encapsidation (initiation) in more detail.

L7 ANSWER 13 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:433036 HCAPLUS
DOCUMENT NUMBER: 125:134569
TITLE: Human immunodeficiency **virus** type 2 **nucleocapsid protein** (NCp7) directs specific initiation of minus-strand DNA synthesis primed by human tRNA3Lys in **vitro**: studies of viral RNA molecules mutated in regions that flank the primer binding site
AUTHOR(S): Li, Xuguang; Quan, Yudong; Arts, Eric J.; Li, Zhuo; Preston, Bradley D.; De Rocquigny, Hugues; Roques, Bernard P.; Darlix, Jean-Luc; Kleiman, Lawrence; et al.
CORPORATE SOURCE: Dep. Med and Microbiology, McGill University AIDS Center, Montreal, QC, Can.
SOURCE: Journal of Virology (1996), 70(8), 4996-5004 CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Retroviral reverse transcription starts near the 5' end of unspliced viral RNA at a sequence called the primer binding site (PBS), where the **tRNA** primer anneals to the RNA template for initiation of DNA synthesis. We have investigated the roles of NCp7 in annealing of primer tRNA3Lys to the PBS and in reverse transcriptase (RT) activity, using a cell-free reverse transcription reaction mixt. consisting of various 5' viral RNA templates, natural primer tRNA3Lys or synthetic primer, human immunodeficiency **virus** type 1 (HIV-1) **nucleocapsid protein** (NCp7), and HIV-1 RT. In the presence of tRNA3Lys, NCp7 was found to stimulate synthesis of minus-strand strong-stop DNA [(-)ssDNA], consistent with previous reports. However, specific DNA synthesis was obsd. only at a NCp7/RNA ratio similar to that predicted to be present in virions. Moreover, at these concns., NCp7 inhibited the synthesis of nonspecific reverse-transcribed DNA products, which are initiated because of self-priming by RNa templates. In contrasts to results obtained with tRNA3Lys as primer, NCp7 inhibited the synthesis of (-)ssDNA products primed by an 18-nucleotide (nt) ribonucleotide (rPR), complementary to the PBS, even through rPR can initiate synthesis of such material in the absence of preannealing with NCp7. Primer placement band shift assays showed that NCp7 was necessary for efficient formation of the **tRNA**-RNA complex. In

contrast, NCp7 was found to prevent formation of the rPR-RNA complex. Since NCp7 appears to exert opposite effects (annealing vs. dissocn.) on tRNA3Lys and rPR substrates, the non-PBS binding regions of the tRNA3Lys mol. may play a role in the annealing of **tRNA** to the template. We also investigated the roles of an A-rich loop upstream of the PBS, a 7-nt region immediately downstream of the PBS, and a 54-nt region that may prevent formation of the U5-leader stem prevented tRNA3Lys placement and priming, while deletions in the A-rich loop or the 7-nt sequence had relatively minor effects in this regard.

L7 ANSWER 14 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:358081 HCAPLUS
DOCUMENT NUMBER: 125:28608
TITLE: RNA binding properties of core protein of the flavivirus Kunjin
AUTHOR(S): Khromykh, A. A.; Westaway, E. G.
CORPORATE SOURCE: Sir Albert Sakzewski Virus Res. Cent., R. Children's Hosp., Brisbane, Australia
SOURCE: Archives of Virology (1996), 141(3-4), 685-699
CODEN: ARVIDF; ISSN: 0304-8608
PUBLISHER: Springer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Kunjin virus (KUN) C is a typical flavivirus core protein which is truncated in vivo to a mature form of 105 residues enriched in lysine and arginine. In order to study the possible assocn. of KUN C with RNA in **vitro**, several recombinant C proteins with specific deletions were prepd., each fused at the N-terminus to glutathione-S-transferase (GST) and expressed in Escherichia coli. They were reacted with KUN RNA probes transcribed in **vitro** from cDNA representing the 5' untranslated region (5' UTR, 93 of 96 nucleotides), the 3' UTR (624 nucleotides), and the 5' UTR plus most of the C coding region (5' core, 440 nucleotides). Fusion protein C107 (incorporating mature C) bound strongly to all KUN RNA probes with apparent specificity, being completely resistant to inhibition by 800 mM NaCl, and to competition by a large excess of **tRNA** . In reactions with labeled KUN RNA probes putative binding sites were identified in the isolated N-terminal (32 residues) and C-terminal (26 residues) basic amino acid domains; this binding was strongly competed by unlabeled KUN UTR probes but weakly or not at all by **tRNA**. These small domains probably acted cooperatively in binding of mature C to KUN RNA probes. The KUN RNA-core protein binding reactions are similar to those reported with other **viral** coat or **capsid proteins** and **viral** RNAs.

L7 ANSWER 15 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:331981 HCAPLUS
DOCUMENT NUMBER: 122:99989
TITLE: Binding of the HIV-1 nucleocapsid protein to the primer tRNA3Lys, in **vitro**, is essentially not specific
AUTHOR(S): Mely, Yves; de Rocquigny, Hugues; Sorinas-Jimeno, Monica; Keith, Gerard; Roques, Bernard P.; Marquet, Roland; Gerard, Dominique
CORPORATE SOURCE: Fac. Pharmacie, Univ. Louis Pasteur Strasbourg I, Illkirch, F-67401, Fr.

SOURCE: Journal of Biological Chemistry (1995), 270(4), 1650-6
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus**, type 1, is a key component in the viral life cycle. Since, the first common step of all reported NCp7 activities corresponds to a nucleic acid-binding step, the NCp7 binding parameters to the natural primer tRNA3Lys were investigated. Using NCp7 intrinsic fluorescence, the authors found that (i) in 0.1 M NaCl, NCp7 bound noncooperatively to tRNA3Lys with a Kobs = 3.2.times.106 M-1 assocn. constant and a binding site size, (ii) four ionic interactions were formed in the NCp7.tRNA3Lys complex, and (iii) nonelectrostatic factors provided about 60% of the binding energy. These binding parameters were not significantly altered when the natural tRNA3Lys was replaced by either an in **vitro** synthetic tRNA3Lys transcript, the heterologous yeast tRNAPhe or the structurally unrelated 5 S RNA from Escherichia coli. Moreover, the environment of the intrinsic fluorescent reporters (Trp37 and Trp61) was similar in the various complexes. Finally, expts. performed at low protein concn. provide no evidence of high affinity binding sites. Taken together, the data strongly suggested an essentially nonspecific binding of NCp7 to tRNA3Lys and thus did not seem to support a direct role of NCp7, per se, in the selection of tRNA3Lys from the pool of cellular **tRNAs**.

L7 ANSWER 16 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:698950 HCAPLUS
DOCUMENT NUMBER: 121:298950
TITLE: Monoclonal antibody-mediated inhibition of RNA binding and annealing activities of HIV type 1 nucleocapsid protein
AUTHOR(S): Tanchou, Valerie; Delaunay, Thierry; Rocquigny, Hugues De; Bodeus, Monique; Darlix, Jean-Luc; Roques, Bernard; Benarous, Richard
CORPORATE SOURCE: Institut Cochin de Genetique Moleculaire, Universite Rene Descartes, Paris, 75014, Fr.
SOURCE: AIDS Research and Human Retroviruses (1994), 10(8), 983-93
CODEN: ARHRE7; ISSN: 0889-2229
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Retroviral nucleocapsid (NC) proteins are highly basic, with one or two zinc fingers, and are required for virion formation, genomic RNA dimerization and packaging, and replication primer **tRNA** annealing to the viral RNA. The authors report here the first characterization of monoclonal antibodies directed against a retroviral nucleocapsid protein and their use to study the structure-function relationships of the nucleocapsid protein NCp7 of HIV-1. Four anti-NCp7 monoclonal antibodies (MAbs) have been generated by using NCp7 of HIV-1. The epitope targets of these MAbs were mapped using ELISA and BIAcore techniques. Whereas three of them are specific for epitopes located in the N and C termini of NCp7, the fourth one appears to be conformation specific. Only two of these MAbs, the conformation-specific one and the MAb recognizing

an N-terminal epitope are able to inhibit the RNA-binding and annealing activities of NCp7 as well as strong-stop DNA synthesis in **vitro**. The binding of the two other MAbs onto NCp7 either has no effect or enhances the NCp7-RNA interactions. These MAbs also display a differential recognition of the Gag polyprotein precursor, which makes them useful tools for studying NC protein maturation in the course of virion morphogenesis.

L7 ANSWER 17 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:673837 HCAPLUS
DOCUMENT NUMBER: 121:273837
TITLE: Amino acid requirements of the nucleocapsid protein of HIV-1 for increasing catalytic activity of a Ki-ras ribozyme in **vitro**
AUTHOR(S): Mueller, Gerd; Strack, Bettina; Dannull, Jens; Sproat, Brian S.; Surovoy, Andrej; Jung, Gunther; Moelling, Karin
CORPORATE SOURCE: Max-Planck-Inst. Molekulare Genetik, Berlin, D-14195, Germany
SOURCE: Journal of Molecular Biology (1994), 242(4), 422-9
CODEN: JMOBAK; ISSN: 0022-2836
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The nucleocapsid protein NCp7 of HIV-1 is a single-stranded nucleic acid binding protein with several functions such as specific recognition, dimerization and packaging of viral RNA, **tRNA** annealing to viral RNA and protection against nucleases. Since some of these functions involve annealing and double-stranded RNA-melting activity we applied the nucleocapsid protein to a hammerhead ribozyme specific for the activated Ki-ras mRNA in **vitro**, which carries at its mutated codon 12 a GUU site. A synthetic ribozyme contg. 2'-O-allyl-modified nucleotides and alternatively in **vitro** transcribed ribozymes were used. At a one to one molar ratio of substrate to ribozyme almost no cleavage is obsd. at 37.degree.. Presence of a synthetic nucleocapsid protein significantly increases the catalytic activity of the ribozyme. Kinetic analyses by means of single and multiple turnover reactions performed at various substrate to ribozyme ratios lead to only a slight stimulation of the rate consts. for single turnover reactions. The rate consts. in multiple turnover reactions, however, are stimulated up to 17-fold by the presence of the nucleocapsid protein. The activating region of the nucleocapsid protein was characterized by a no. of mutants. The mutants demonstrate that activation requires both basic amino acid clusters as evidenced by point mutations. Deletion mutants indicate that the second zinc finger is totally dispensable and that replacement of the first zinc finger by a glycine-glycine spacer only slightly reduces the enhancing effect of the nucleocapsid protein on the ribozyme.

L7 ANSWER 18 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:466114 HCAPLUS
DOCUMENT NUMBER: 119:66114
TITLE: Identification of a binding site for the human immunodeficiency **virus** type 1 **nucleocapsid protein**

AUTHOR(S): Sakaguchi, Kazuyasu; Zambrano, Nicola; Baldwin, Eric T.; Shapiro, Bruce A.; Erickson, John W.; Omichinski, James G.; Clore, G. Marius; Gronenborn, Angela M.; Appella, Ettore
CORPORATE SOURCE: Lab. Cell Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1993), 90(11), 5219-23
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The nucleocapsid (NC) protein NCp7 of human immunodeficiency virus type 1 (HIV-1) is important for encapsidation of the virus genome, RNA dimerization, and primer **tRNA** annealing in **vitro**. Here evidence from gel mobility-shift expts. is presented indicating that NCp7 binds specifically to an RNA sequence. Two complexes were identified in native gels. The more slowly migrating complex contained two RNA mols. and one peptide, while the more rapidly migrating one is composed of one RNA and one peptide. Further, mutational anal. of the RNA shows that the predicted stem and loop structure of stem-loop 1 plays a crit. role. These results show that NCp7 binds to a unique RNA structure within the .psi. region; in addn., this structure is necessary for RNA dimerization. It is proposed that NCp7 binds to the RNA via a direct interaction of one zinc-binding motif to stem-loop 1 followed by binding of the other zinc-binding motif to stem-loop 1, stem-loop 2, or the linker region of the second RNA mol., forming a bridge between the two RNAs.

L7 ANSWER 19 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:229970 HCAPLUS
DOCUMENT NUMBER: 118:229970
TITLE: RNA binding of recombinant nucleocapsid proteins of hantaviruses
AUTHOR(S): Goett, Peter; Stohwasser, Ralf; Schnitzler, Paul; Darai, Gholamreza; Bautz, Ekkehard K. F.
CORPORATE SOURCE: ZMBH, Univ. Heidelberg, Heidelberg, 6900, Germany
SOURCE: Virology (1993), 194(1), 332-7
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Genes encoding the nucleocapsid (N) proteins of two hantaviruses, Hantaan virus strain 76-118 (HTN) and Puumala virus strain CG 18-20 (PUU), were expressed in Escherichia coli as histidine-tagged proteins. They were purified by metal-chelate affinity chromatog. under native or denaturing conditions to near homogeneity. The sol. form of HTN N protein was assocd. with RNA of E. coli. Renatured N proteins were shown to bind in **vitro** transcribed RNA representing the hantaviral small genomic (S) RNA segment. RNA binding was shown by affinity to filter-immobilized N proteins and by gel mobility shift assays. Competition expts. using **tRNA**, poly(U) and poly(A)+ U indicated that binding of RNA by the N protein is nonspecific. However, direct binding of dsRNA resulted in efficient formation of large complexes suggesting that double-stranded nucleic acids are bound preferentially. Carboxyterminal fragments of HTN and PUU N proteins contg. about 100

amino acids of the carboxy termini retained full binding capacity indicating that RNA binding occurs via a carboxyterminal domain.

L7 ANSWER 20 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1991:530209 HCAPLUS
DOCUMENT NUMBER: 115:130209
TITLE: **Viral** RNA annealing activities of the **nucleocapsid protein** of Moloney murine leukemia **virus** are zinc independent
AUTHOR(S): Prats, Anne Catherine; Housset, Valerie; De Billy, Gerard; Cornille, Fabrice; Prats, Herve; Roques, Bernard; Darlix, Jean Luc
CORPORATE SOURCE: Cent. Rech. Biochim. Genet. Cell., CNRS, Toulouse, 31062, Fr.
SOURCE: Nucleic Acids Research (1991), 19(13), 3533-41
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The zinc fingers of retroviral gag nucleocapsid proteins (NC) are required for the specific packaging of the dimeric RNA genome into virions. In **vitro**, NC proteins activate both dimerization of viral RNA and annealing of the replication primer **tRNA** onto viral RNA, two reactions necessary for the prodn. of infectious virions. In this study the role of the zinc finger of Moloney murine leukemia virus (MoMuLV) NCp10 in RNA binding and annealing activities was investigated through modification or replacement of residues involved in zinc coordination. These alterations did not affect the ability of NCp10 to bind RNA and promote RNA annealing in **vitro**, despite a complete loss of zinc affinity. However mutation of two conserved lysine residues adjacent to the finger motif reduced both RNA binding and annealing activities of NCp10. These findings suggest that the complexed NC zinc finger is not directly involved in RNA-protein interactions but more probably in a zinc dependent conformation of NC protein modulating viral protein-protein interactions, essential to the process of viral RNA selection and virion assembly. Then the NC zinc finger may cooperate to select the viral RNA genome to be packaged into virions.

L7 ANSWER 21 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1989:73871 HCAPLUS
DOCUMENT NUMBER: 110:73871
TITLE: Cell-free system for protein formation from template mRNA containing ribosomes, amino acids, ATP, and GTP
INVENTOR(S): Alakhov, Yu. B.; Baranov, V. I.; Ovodov, S. Yu.; Ryabova, L. A.; Spirin, A. S.
PATENT ASSIGNEE(S): Institute of Protein, Academy of Sciences, U.S.S.R., USSR
SOURCE: PCT Int. Appl., 19 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Russian
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 8808453	A1	19881103	WO 1988-SU78	19880414
W: BG, FI, HU, JP, US				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
SU 1441787	A1	19900923	SU 1987-4239148	19870429
EP 312617	A1	19890426	EP 1988-904712	19880414
EP 312617	B1	19930303		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
JP 01503119	T2	19891026	JP 1988-504206	19880414
JP 07110236	B4	19951129		
HU 54420	A2	19910228	HU 1988-3398	19880414
AT 86306	E	19930315	AT 1988-904712	19880414
FI 8806002	A	19881228	FI 1988-6002	19881228
PRIORITY APPLN. INFO.:			SU 1987-4239148	19870429
			EP 1988-904712	19880414
			WO 1988-SU78	19880414

AB A cell-free system contg. ATP, GTP, and amino acids is described for the in **vitro** translation of mRNA to obtain, in high yield, proteins contg. 15-70 amino acids. The system contains ATP, GTP, 20 free amino acids, the mRNA to be translated, and ribosomes (e.g. from Escherichia coli or wheat embryo ext.). The mRNA for the capsid protein of phage MS2 was translated in a cell-free system contg. E. coli ribosomes in the presence of ATP, GTP, MS2 **tRNA**, and 20 amino acids. After 20 min incubation at 37.degree. the E. coli ribosomes synthesized 6000 pmol capsid protein (100 pmol cellular product/pmol capsid mRNA). Addn. of salmon calcitonin mRNA to the translation system resulted in formation of 18,000 pmol calcitonin (300 pmol cellular prooduct/pmol calcitonin mRNA). Optionally, wheat germ ext. was used in place of E. coli ribosomes to translate brome mosaic **virus** (BMV) **capsid protein** mRNA. After 20 h incubation 10,000 pmol BMV capsid protein was recovered (100 pmol cell product/pmol BMV mRNA). When salmon calcitonin mRNA was added to this system 15,000 pmol calcitonin was recovered (150 pmol cell product/pmol calcitonin mRNA).

L7 ANSWER 22 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1987:152484 HCAPLUS
DOCUMENT NUMBER: 106:152484
TITLE: Translation of homologous and heterologous messenger RNAs in a yeast cell-free system
AUTHOR(S): Hussain, Iffat; Leibowitz, Michael J.
CORPORATE SOURCE: Robert Wood Johnson Med. Sch., UMDNJ, Piscataway, NJ, 08854, USA
SOURCE: Gene (1986), 46(1), 13-23
CODEN: GENED6; ISSN: 0378-1119
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A stable mRNA-dependent cell-free translation system from Saccharomyces cerevisiae, prepd. by a modification of the method of R. Hofbauer et al. (1982) was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addn. of polyamines and yeast **tRNA**. The m transcript of the M segment of double-stranded RNA, synthesized in **vitro** using the killer virus-assocd. RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunol. identified using antitoxin antibody. Sindbis **virus** **capsid protein** and rabbit globin were also

translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin, and anisomycin. Analogs of the 5'-terminal caps present on most eukaryotic mRNA mols. inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

L7 ANSWER 23 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1986:46420 HCAPLUS
DOCUMENT NUMBER: 104:46420
TITLE: Direct mapping of adeno-associated **virus capsid proteins** B and C: a possible ACG initiation codon
AUTHOR(S): Becerra, S. Patricia; Rose, James A.; Hardy, Medora; Baroudy, Bahige M.; Anderson, Carl W.
CORPORATE SOURCE: Lab. Biol. Viruses, Natl. Inst. Allergy Infect. Dis., Bethesda, MD, 20205, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1985), 82(23), 7919-23
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 3 major **capsid proteins** of adeno-assocd. **virus** type 2 (AAV2) virions are designated A, B, and C and have mol. sizes of 90, 72, and 60 kDa, resp. These proteins are related, and genetic studies have shown that they are encoded by a long open reading frame located in the right half of the genome. The coding capacity distal to the 1st ATG in this reading frame is only 503 amino acids (i.e., a protein about the size of protein C), but an open frame sequence devoid of ATG codons extends upstream for an addnl. 184 codons. Although the N terminus of the C capsid protein is blocked, partial amino acid sequence analyses of peptides from C have confirmed that it is encoded within the portion of the reading frame distal to the 1st ATG at nucleotide (nt) location 2810. The N terminus of the B capsid protein is not blocked, and its sequence begins with alanine. The triplet encoding this alanine lies 64 codons upstream from the initiation site for protein C and is immediately preceded by the threonine codon, ACG, at nucleotide 2615. This ACG codon lies in the most favorable sequence context for protein synthesis initiation. All 3 AAV2 capsid proteins are labeled in **vitro** with formyl[35S]methionyl-tRNAf, indicating that synthesis of each protein is initiated independently. Apparently, the nucleotide 2615 ACG codon directs the methionyl-**tRNA**-dependent initiation of the AAV2B capsid protein. Proteins B and C may be synthesized from the same mRNA species, and their relative abundance could be detd. by the efficiencies of their resp. initiation codons.

L7 ANSWER 24 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1978:543231 HCAPLUS
DOCUMENT NUMBER: 89:143231
TITLE: Polyoma virus has three late mRNA's: one for each virion protein
AUTHOR(S): Siddell, Stuart G.; Smith, Alan E.
CORPORATE SOURCE: Transl. Lab., Imperial Cancer Res. Fund, London, Engl.
SOURCE: J. Virol. (1978), 27(2), 427-31
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal

LANGUAGE: English
AB Polyoma virus mRNA, from the cytoplasm of 3T6 cells late after infection and purified by hybridization to HpaII fragment 3 of polyoma virus DNA, was sepd. on 50% formamide-contg. sucrose d. gradients, and the fractionated RNA was recovered and translated in **vitro**. Anal. of the cell-free products showed the minor virion protein VP3 was synthesized from an mRNA sedimenting at .apprx.18S between the 19S VP2 mRNA and the 16S VP1 mRNA. The VP2 and VP3 could be labeled with formylmethionine from initiator **tRNA**. Apparently there are 3 late polyoma virus mRNAs, each directing the synthesis of only 1 **viral capsid protein**.

L7 ANSWER 25 OF 25 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1976:556399 HCAPLUS
DOCUMENT NUMBER: 85:156399
TITLE: Initiation of translation directed by 42S and 26S RNAs from Semliki Forest virus in **vitro**
AUTHOR(S): Glanville, Niall; Ranki, Marjut; Morser, John; Kaariainen, Leevi; Smith, Alan E.
CORPORATE SOURCE: Dep. Virol., Univ. Helsinki, Helsinki, Finland
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1976), 73(9), 3059-63
CODEN: PNASA6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The proteins synthesized in **vitro** in response to 42S and 26S RNAs from Semliki Forest virus were labeled with formylmethionine-35S from initiator **tRNA**. One **protein** which comigrated with **viral capsid protein** was labeled under the direction of 26S RNA, and only 1 labeled peptide was detected after digestion with trypsin. Further digestion with Pronase gave rise to the dipeptide formylmethionine-asparagine. Several labeled polypeptides were found in the 42S RNA directed product and these had mol. wts. of up to 150,000. However, tryptic digestion of the product yielded only 1 formylmethionyl-labeled peptide, which had a different mobility from that directed by the 26S RNA. Further digestion with Pronase gave a single dipeptide, formylmethionine-alanine. This indicates that nonstructural proteins as large as 150,000 daltons are probably synthesized from 1 initiation site on the 42S RNA. Translation starting from the internal initiation site on the 42S RNA, which is equiv. to that on the 26S RNA, could not be detected under the conditions used. Internal initiation sites which are similarly inactive have also been detected in other viral RNAs (e.g., brome mosaic virus, tobacco mosaic virus, and polyoma 19S RNA) and this suggests that, although eukaryotic mRNAs can contain more than 1 initiation site for protein synthesis, only the site nearer the 5' terminus is active in **vitro**.

'FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 12:39:02 ON 08 OCT 2002)
L8 100 S L7
L9 44 DUP REM L8 (56 DUPLICATES REMOVED)

L9 ANSWER 1 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2002:278978 SCISEARCH

THE GENUINE ARTICLE: 533GG
TITLE: The Tat protein of human immunodeficiency virus type 1 (HIV-1) can promote placement of **tRNA** primer onto viral RNA and suppress later DNA polymerization in HIV-1 reverse transcription
AUTHOR: Kameoka M; Morgan M; Binette M; Russell R S; Rong L W; Guo X F; Mouland A; Kleiman L; Liang C; Wainberg M A (Reprint)
CORPORATE SOURCE: McGill Univ, Jewish Gen Hosp, AIDS Ctr, Lady Davis Inst, 3755 Cote Ste Catherine Rd, Montreal, PQ H3T 1E2, Canada (Reprint); McGill Univ, Jewish Gen Hosp, AIDS Ctr, Lady Davis Inst, Montreal, PQ H3T 1E2, Canada; McGill Univ, Dept Microbiol, Montreal, PQ H3A 2B4, Canada
COUNTRY OF AUTHOR: Canada
SOURCE: JOURNAL OF VIROLOGY, (APR 2002) Vol. 76, No. 8, pp. 3637-3645.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 51

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human immunodeficiency virus type-1 Tat has been proposed to play a role in the regulation of reverse transcription. We previously demonstrated that wild-type Tat can augment viral infectivity by suppressing the reverse transcriptase (RT) reaction at late stages of the viral life cycle in order to prevent the premature synthesis of potentially deleterious viral DNA products. Here we have performed a detailed analysis of the cell-free reverse transcription reaction to elucidate the mechanism(s) whereby Tat can affect this process. Our results show that Tat can suppress nonspecific DNA elongation while moderately affecting the specific initiation stage of reverse transcription. In addition, Tat has an RNA-annealing activity and can promote the placement of **tRNA** onto viral RNA. This points to a functional homology between Tat and the **viral nucleocapsid** (NC) **protein** that is known to be directly involved in this process. Experiments using a series of mutant Tat proteins revealed that the cysteine-rich and core domains of Tat are responsible for suppression of DNA elongation, while each of the cysteine-rich, core, and basic domains, as well as a glutamine-rich region in the C-terminal domain, are important for the placement of **tRNA** onto the viral RNA genome. These results suggest that Tat can play at least two different roles in the RT reaction, i.e., suppression of DNA polymerization and placement of **tRNA** onto viral RNA. We believe that the first of these activities of Tat may contribute to the overall efficiency of reverse transcription of the viral genome during a new round of infection as well as to enhanced production of infectious viral particles. We hypothesize that the second activity, illustrating functional homology between Tat and NC, suggests a potential role for NC in the displacement of Tat during viral maturation.

L9 ANSWER 2 OF 44 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002311005 MEDLINE
DOCUMENT NUMBER: 22050818 PubMed ID: 12054775

TITLE: Functional interactions of **nucleocapsid protein** of feline immunodeficiency **virus** and cellular prion **protein** with the viral RNA.
AUTHOR: Moscardini Mila; Pistello Mauro; Bendinelli M; Ficheux Damien; Miller Jennifer T; Gabus Caroline; Le Grice Stuart F J; Surewicz Witold K; Darlix Jean-Luc
CORPORATE SOURCE: Department of Biomedicine, University of Pisa, I-56127 Pisa, Italy.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2002 Apr 19) 318 (1) 149-59.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020611
Last Updated on STN: 20020713
Entered Medline: 20020712

AB All lentiviruses and oncoretroviruses examined so far encode a major nucleic-acid binding protein (nucleocapsid or NC* protein), approximately 2500 molecules of which coat the dimeric RNA genome. Studies on HIV-1 and MoMuLV using in **vitro** model systems and in vivo have shown that NC protein is required to chaperone viral RNA dimerization and packaging during virus assembly, and proviral DNA synthesis by reverse transcriptase (RT) during infection. The human cellular prion protein (PrP), thought to be the major component of the agent causing transmissible spongiform encephalopathies (TSE), was recently found to possess a strong affinity for nucleic acids and to exhibit chaperone properties very similar to HIV-1 NC protein in the HIV-1 context in **vitro**. Tight binding of PrP to nucleic acids is proposed to participate directly in the prion disease process. To extend our understanding of lentiviruses and of the unexpected nucleic acid chaperone properties of the human prion protein, we set up an in **vitro** system to investigate replication of the feline immunodeficiency virus (FIV), which is functionally and phylogenetically distant from HIV-1. The results show that in the FIV model system, NC protein chaperones viral RNA dimerization, primer **tRNA**(Lys,3) annealing to the genomic primer-binding site (PBS) and minus strand DNA synthesis by the homologous FIV RT. FIV NC protein is able to trigger specific viral DNA synthesis by inhibiting self-priming of reverse transcription. The human prion protein was found to mimic the properties of FIV NC with respect to primer **tRNA** annealing to the viral RNA and chaperoning minus strand DNA synthesis.

L9 ANSWER 3 OF 44 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000270264 MEDLINE
DOCUMENT NUMBER: 20270264 PubMed ID: 10809779
TITLE: In **vitro** studies on **tRNA** annealing and reverse transcription with mutant HIV-1 RNA templates.
AUTHOR: Beerens N; Berkhout B
CORPORATE SOURCE: Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Amsterdam 1100 DE,

The Netherlands.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 May 19) 275 (20) 15474-81.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000629
Last Updated on STN: 20000629
Entered Medline: 20000621

AB The human immunodeficiency virus type 1 (HIV-1) RNA genome encodes a semistable stem-loop structure, the U5-PBS hairpin, which occludes part of the **tRNA** primer binding site (PBS). In previous studies, we demonstrated that mutations that alter the stability of the U5-PBS hairpin inhibit virus replication. A reverse transcription defect was measured in assays with the virion-extracted RNA-**tRNA** complexes. We now extend these studies with in **vitro** synthesized wild-type and mutant RNA templates that were tested in primer annealing and reverse transcription assays. The effect of annealing temperature and the presence of the **viral nucleocapsid protein** on reverse transcription was analyzed for the templates with a stabilized or destabilized U5-PBS hairpin, and in reactions initiated by **tRNA** or DNA primers. The results of this in **vitro** assay are consistent with the in vivo findings, in that both **tRNA** annealing and initiation of reverse transcription are sensitive to stable template RNA structure. Reverse transcription initiated by a DNA primer is less hindered by secondary structure in the RNA template than **tRNA** primed reactions. The inhibitory effect of template structure on **tRNA**-primed reverse transcription is more pronounced in this in **vitro** assay compared with the in vivo material, indicating that the heat-annealed RNA-**tRNA** complex differs from the virion-extracted viral RNA-**tRNA** complex.

L9 ANSWER 4 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2000:703994 SCISEARCH
THE GENUINE ARTICLE: 352XH
TITLE: Zinc finger structures in the human immunodeficiency **virus** type 1 **nucleocapsid protein** facilitate efficient minus- and plus-strand transfer
AUTHOR: Guo J H; Wu T Y; Anderson J; Kane B F; Johnson D G; Gorelick R J; Henderson L E; Levin J G (Reprint)
CORPORATE SOURCE: NICHHD, MOL GENET LAB, NIH, BLDG 6B, RM 216, BETHESDA, MD 20892 (Reprint); NICHHD, MOL GENET LAB, NIH, BETHESDA, MD 20892; NCI, AIDS VACCINE PROGRAM, SAIC, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF VIROLOGY, (OCT 2000) Vol. 74, No. 19, pp. 8980-8988.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0022-538X.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 83
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **nucleocapsid protein** (NC) of human immunodeficiency **virus** type 1 (HIV-1) has two zinc fingers, each containing the invariant metal ion binding residues CCHC, Recent reports indicate that mutations In the CCHC motifs are deleterious for reverse transcription in vivo. To identify reverse transcriptase (RT) reactions affected by such changes, cre have probed zinc finger functions in NC-dependent RT-catalyzed HIV-1 minus- and plus-strand transfer model systems. Our approach was to examine the activities of wild-type NC and a mutant in which all six cysteine residues were replaced by serine (SSHS NC); this mutation severely disrupts zinc coordination. We find that the zinc fingers contribute to the role of NC in complete **tRNA** primer removal from minus-strand DNA during plus-strand transfer, Annealing of the primer binding site sequences in plus-strand strong-stop DNA [(+) SSDNA] to its complement in minus-strand acceptor DNA is not dependent on SC zinc fingers. In contrast, the rate of annealing of the complementary R regions in (-) SSDNA, and 3' viral RNA during minus-strand transfer is approximately eightfold lower when SSHS NC is used in place of wild-type NC. Moreover, unlike wild-type NC, SSHS SC has only a small stimulatory effect on minus-strand transfer and is essentially unable to block TAR-induced self-priming from (-) SSDNA. Our results strongly suggest that NC zinc finger structures are needed to unfold highly structured RNA and DNA strand transfer intermediates. Thus, it appears that in these cases, zinc finger interactions are important components of NC nucleic acid chaperone activity.

L9 ANSWER 5 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2000:703972 SCISEARCH
THE GENUINE ARTICLE: 352XH
TITLE: Human immunodeficiency **virus** type 1 **nucleocapsid protein** can prevent self-priming of minus-strand strong stop DNA by promoting the annealing of short oligonucleotides to hairpin sequences
AUTHOR: Driscoll M D; Hughes S H (Reprint)
CORPORATE SOURCE: NCI, HIV DRUG RESISTANCE PROGRAM, FREDERICK CANC RES & DEV CTR, POB B, FREDERICK, MD 21702 (Reprint); NCI, ABL BASIC RES PROGRAM, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF VIROLOGY, (OCT 2000) Vol. 74, No. 19, pp. 8785-8792.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 41
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Understanding how viral components collaborate to convert the human immunodeficiency virus type 1 genome from single-stranded RNA

into double-stranded DNA is critical to the understanding of viral replication. Not only must the correct reactions be carried out, but unwanted side reactions must be avoided. After minus-strand strong stop DNA (-sssDNA) synthesis, degradation of the RNA template by the RNase Il domain of reverse transcriptase (RT) produces single-stranded DNA that has the potential to self-prime at the imperfectly base paired TAR hairpin, making continued DNA synthesis impossible. Although nucleocapsid protein (NC) interferes with -sssDNA self-priming in reverse transcription reactions in **vitro**, NC alone did not prevent self-priming of a synthetic -sssDNA oligomer. NC did not influence DNA bending and therefore cannot inhibit self-priming at hairpins by directly blocking hairpin formation. Using DNA oligomers as a model for genomic RNA fragments, we found that a 17-base DNA fragment annealed to the 3' end of the -sssDNA prevented self priming in the presence of NC. This implies that to avoid self-priming, an RNA-DNA hybrid that is more thermodynamically stable than the hairpin must remain within the hairpin region. This suggests that NC prevents self-priming by generating or stabilizing a thermodynamically favored RNA-DNA heteroduplex instead of the kinetically favored TAR hairpin. In support of this idea, sequence changes that increased base pairing in the DNA TAR hairpin resulted in an increase in self-priming in **vitro**. We present a model describing the role of NC-dependent inhibition of self-priming in first-strand transfer.

L9 ANSWER 6 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2000:265549 SCISEARCH
THE GENUINE ARTICLE: 299TW
TITLE: Interactions between human immunodeficiency virus type 1 reverse transcriptase, **tRNA** primer, and nucleocapsid protein during reverse transcription
AUTHOR: Hsu M; Wainberg M A (Reprint)
CORPORATE SOURCE: MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, 3755 CH COTE STE CATHERINE, MONTREAL, PQ H3T 1E2, CANADA (Reprint); MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA; MCGILL UNIV, DEPT MICROBIOL & IMMUNOL, MONTREAL, PQ H3T 1E2, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: JOURNAL OF HUMAN VIROLOGY, (JAN-FEB 2000) Vol. 3, No. 1, pp. 16-26.
Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621.
ISSN: 1090-9508.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 108

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An early step in the life cycle of human immunodeficiency virus type 1 is the reverse transcription of the viral RNA genome into double-stranded DNA, which is subsequently translocated to the cell nucleus. It is then integrated into host DNA and serves as a template for viral gene expression. Reverse transcription is catalyzed by the viral enzyme reverse transcriptase and is a complex process comprising a series of RNA-dependent DNA polymerization, DNA-dependent DNA polymerization. and RNase H reactions. Strand transfer reactions are required to complete the process. Re-verse

transcription is initiated when a molecule of host cell **tRNA** (lys3). which serves as a primer, is bound to the primer binding site of **viral** genomic RNA. The **viral** **nucleocapsid protein** is involved in each of the initiation of reverse transcription and in subsequent strand transfer or template-switching events. We review the interactions among reverse transcriptase, viral genomic RNA, the **tRNA** primer of reverse transcription, and **viral** **nucleocapsid protein** in the various steps of reverse transcription, including primer placement, initiation, and processive synthesis.

L9 ANSWER 7 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1999:307393 SCISEARCH
THE GENUINE ARTICLE: 186VV
TITLE: Evidence of interactions between the nucleocapsid protein NCp7 and the reverse transcriptase of HIV-1
AUTHOR: Druillennec S; Caneparo A; deRocquigny H; Roques B P (Reprint)
CORPORATE SOURCE: UFR SCI PHARMACEUT & BIOL 4, DEPT PHARMACOCHIM MOL & STRUCT, CNRS, UMR 8600, INSERM, U266, F-75270 PARIS 06, FRANCE (Reprint); UFR SCI PHARMACEUT & BIOL 4, DEPT PHARMACOCHIM MOL & STRUCT, CNRS, UMR 8600, INSERM, U266, F-75270 PARIS 06, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (16 APR 1999) Vol. 274, No. 16, pp. 11283-11288.
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
ISSN: 0021-9258.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The human immunodeficiency **virus** (HIV-1) **nucleocapsid protein** NCp7 containing two CX2CX4HX4C-type zinc fingers was proposed to be involved in reverse transcriptase (RT)-catalyzed proviral DNA synthesis through promotion of **tRNA**(3)(LYS) annealing to the RNA primer binding site, improvement of DNA strand transfers, and enhancement of RT processivity. The NCp7 structural characteristics are crucial because mutations altering the finger domain conformation led to noninfectious viruses characterized by defects in provirus integration. These findings prompted us to study a putative RT/NCp7 protein-protein interaction. Binding as says using far Western analysis or RT immobilized on beads clearly showed the formation of a complex between NCp7 and RT. The affinity of NCp7 for p66/p51RT was 0.60 mu M with a 1:1 stoichiometry. This interaction was confirmed by chemical cross-linking and co-immunoprecipitation of the two proteins in a viral environment. Competition experiments using different NCp7 mutants showed that alteration of the finger structure disrupted RT recognition giving insights into the loss of infectivity of corresponding HIV-1 mutants. Together with structural data on RT, these results suggest that the role of NCp7 could be to enhance RT processivity through stabilization of a p51-induced active form of the p66 subunit and open the way for designing new antiviral agents.

L9 ANSWER 8 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:263770 BIOSIS
DOCUMENT NUMBER: PREV199900263770
TITLE: Papillomavirus capsid protein expression level depends on the match between codon usage and **tRNA** availability.
AUTHOR(S): Zhou, Jian; Liu, Wen Jun; Peng, Shi Wen; Sun, Xiao Yi; Frazer, Ian (1)
CORPORATE SOURCE: (1) Centre for Immunology and Cancer Research, University of Queensland, Princess Alexandra Hospital, Brisbane, QLD, 4102 Australia
SOURCE: Journal of Virology, (June, 1999) Vol. 73, No. 6, pp. 4972-4982.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Translation of mRNA encoding the L1 and L2 capsid proteins of papillomavirus (PV) is restricted in vivo to differentiated epithelial cells, although transcription of the L1 and L2 late genes occurs more widely. The codon composition of PV late genes is quite different from that of most mammalian genes. To test the possibility that PV late gene codon composition determines the efficiency of PV late gene expression in some cell types, synthetic bovine papillomavirus type 1 (BPV1) late genes were constructed with codon composition modified to resemble the typical mammalian gene. Expression of these genes from a strong promoter in Cos-1 cells was compared with expression of wild-type BPV1 late genes from the same promoter. Both unmodified and modified PV late genes were transcribed in Cos-1 cells, but only the codon-modified genes were translated. In **vitro** translation of wild-type but not synthetic BPV1 L1 mRNA was markedly enhanced by addition of aminoacyl-**tRNAs**. Codon composition thus limits BPV1 late gene translation in Cos-1 cells, and this limitation can be overcome by modification of the codon composition of the genes or by provision of excess **tRNA**. Replacement of codons in the green fluorescent protein (gfp) gene with those frequently used in PV late genes did not alter gfp transcription in Cos-1 cells but almost abolished translation, supporting the hypothesis that the observed differences in efficiency of translation of modified and unmodified PV capsid genes were related to codon usage rather than mRNA structure. As **tRNA** populations vary within and between tissues in the same eukaryotic organism, we speculate that matching of **tRNA** availability to codon usage may be one determinant of the restriction of expression of PV late genes to differentiated epithelium.

L9 ANSWER 9 OF 44 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999134376 MEDLINE
DOCUMENT NUMBER: 99134376 PubMed ID: 9933645
TITLE: Role of post-transcriptional modifications of primer tRNALys,3 in the fidelity and efficacy of plus strand DNA transfer during HIV-1 reverse transcription.
AUTHOR: Auxilien S; Keith G; Le Grice S F; Darlix J L
CORPORATE SOURCE: LaboRetro ENS, INSERM U412, 46 allee d'Italie, 69364 Lyon cedex 07, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274

(7) 4412-20.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990311

AB During HIV reverse transcription, (+) strand DNA synthesis is primed by an RNase H-resistant sequence, the polypurine tract, and continues as far as a 18-nt double-stranded RNA region corresponding to the 3' end of tRNALys,3 hybridized to the viral primer binding site (PBS). Before (+) strand DNA transfer, reverse transcriptase (RT) needs to unwind the double-stranded **tRNA**-PBS RNA in order to reverse-transcribe the 3' end of primer tRNALys,3. Since the detailed mechanism of (+) strand DNA transfer remains incompletely understood, we developed an in **vitro** system to closely examine this mechanism, composed of HIV 5' RNA, natural modified tRNALys,3, synthetic unmodified tRNALys,3 or oligonucleotides (RNA or DNA) complementary to the PBS, as well as the **viral proteins** RT and **nucleocapsid protein** (NCp7). Prior to (+) strand DNA transfer, RT stalls at the double-stranded **tRNA**-PBS RNA complex and is able to reverse-transcribe modified nucleosides of natural tRNALys,3. Modified nucleoside m1A-58 of natural tRNALys,3 is only partially effective as a stop signal, as RT can transcribe as far as the hyper-modified adenosine (ms2t6A-37) in the anticodon loop. m1A-58 is almost always transcribed into A, whereas other modified nucleosides are transcribed correctly, except for m7G-46, which is sometimes transcribed into T. In contrast, synthetic tRNALys,3, an RNA PBS primer, and a DNA PBS primer are completely reverse-transcribed. In the presence of an acceptor template, (+) strand DNA transfer is efficient only with templates containing natural tRNALys,3 or the RNA PBS primer. Sequence analysis of transfer products revealed frequent errors at the transfer site with synthetic tRNALys,3, not observed with natural tRNALys,3. Thus, modified nucleoside m1A-58, present in all retroviral **tRNA** primers, appears to be important for both efficacy and fidelity of (+) strand DNA transfer. We show that other factors such as the nature of the (-) PBS of the acceptor template and the RNase H activity of RT also influence the efficacy of (+) strand DNA transfer.

L9 ANSWER 10 OF 44 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999214364 MEDLINE
DOCUMENT NUMBER: 99214364 PubMed ID: 10196321
TITLE: The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of **tRNA** on the primer binding site.
AUTHOR: Feng Y X; Campbell S; Harvin D; Ehresmann B; Ehresmann C; Rein A
CORPORATE SOURCE: Retroviral Genetics Section, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland

21702, USA.
SOURCE: JOURNAL OF VIROLOGY, (1999 May) 73 (5) 4251-6.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199905
ENTRY DATE: Entered STN: 19990601
Last Updated on STN: 19990601
Entered Medline: 19990519

AB The formation of an infectious retrovirus particle requires several RNA-RNA interaction events. In particular, the genomic RNA molecules form a dimeric structure, and a cellular **tRNA** molecule is annealed to an 18-base complementary region (the primer binding site, or PBS) on the genomic RNA, where it will serve as primer for reverse transcription. **tRNAs** normally possess a highly stable secondary and tertiary structure; it seems unlikely that annealing of a **tRNA** molecule to the PBS, which involves unwinding of this structure, could occur efficiently at physiological temperatures without the assistance of a cofactor. Many prior studies have shown that the **viral nucleocapsid** (NC) **protein** can act as a nucleic acid chaperone (i.e., facilitate annealing events between nucleic acids), and the assays used to demonstrate this activity include its ability to catalyze dimerization of transcripts representing retroviral genomes and the annealing of **tRNA** to the PBS in **vitro**. However, mature NC is not required for these events in vivo, since protease-deficient viral mutants, in which NC is not cleaved from the parental Gag polyprotein, are known to contain dimeric RNAs with **tRNA** annealed to the PBS. In the present experiments, we have tested recombinant human immunodeficiency virus type 1 Gag polyprotein for nucleic acid chaperone activity. The protein was positive by all of our assays, including the ability to stimulate dimerization and to anneal **tRNA** to the PBS in **vitro**. In quantitative experiments, its activity was approximately equivalent on a molar basis to that of NC. Based on these results, we suggest that the Gag polyprotein (presumably by its NC domain) catalyzes the annealing of **tRNA** to the PBS during (or before) retrovirus assembly in vivo.

L9 ANSWER 11 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1999:205082 SCISEARCH
THE GENUINE ARTICLE: 173GG
TITLE: Time-resolved fluorescence investigation of the human immunodeficiency **virus** type 1 **nucleocapsid protein**: Influence of the binding of nucleic acids
AUTHOR: Bombarda E; Ababou A; Vuilleumier C; Gerard D; Roques B P; Piemont E; Mely Y (Reprint)
CORPORATE SOURCE: UNIV STRASBOURG 1, FAC PHARM, BIOPHYS LAB, CNRS, URA 491, BP 24, F-67401 ILLKIRCH GRAFFENS, FRANCE (Reprint); UNIV STRASBOURG 1, FAC PHARM, BIOPHYS LAB, CNRS, URA 491, F-67401 ILLKIRCH GRAFFENS, FRANCE; FAC PHARM, CNRS, URA D1500, INSERM, U266, UNITE PHARMACOCHIM MOL, F-75270 PARIS 06, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: BIOPHYSICAL JOURNAL, (MAR 1999) Vol. 76, No. 3, pp.

1561-1570.
Publisher: BIOPHYSICAL SOCIETY, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
ISSN: 0006-3495.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Depending on the HIV-1 isolate, MN or BH10, the nucleocapsid protein, NCp7, corresponds to a 55- or 71-amino acid length product, respectively. The MN NCp7 contains a single Trp residue at position 37 in the distal zinc finger motif, and the BH10 NCp7 contains an additional Trp, at position 61 in the C-terminal chain. The time-resolved intensity decay parameters of the zinc-saturated BH10 NCp7 were determined and compared to those of single-Trp-containing derivatives. The fluorescence decay of BH10 NCp7 could be clearly represented as a linear combination (with respect to both lifetimes and fractional intensities) of the individual emitting Trp residues. This suggested the absence of interactions between the two Trp residues, a feature that was confirmed by molecular modeling and fluorescence energy transfer studies. In the presence of **tRNA**(Phe), taken as a RNA model, the same conclusions hold true despite the large fluorescence decrease induced by the binding of **tRNA**(Phe). Indeed, the fluorescence of Trp(37) appears almost fully quenched, in keeping with a stacking of this residue with the bases of **tRNA**(Phe). Despite the multiple binding sites in **tRNA**(Phe), the large prevalence of ultrashort lifetimes, associated with the stacking of Trp(37), suggests that this stacking constitutes a major feature in the binding process of NCp7 to nucleic acids. In contrast, Trp(61) only stacked to a small extent with tRNAPhe. Th, behavior of this residue in the **tRNA**(Phe)-NCp7 complexes appeared to be rather heterogeneous, suggesting that it does not constitute a major determinant in the binding process. Finally, our data suggested that the binding of NCp7 proteins from the two HIV-1 strains to nonspecific nucleic acid sequences was largely similar.

L9 ANSWER 12 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1999:87029 BIOSIS
DOCUMENT NUMBER: PREV199900087029

TITLE: Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus in **vitro**.

AUTHOR(S): Sasaki, Jun; Nakashima, Nobuhiko (1)
CORPORATE SOURCE: (1) Natl. Inst. Sericultural Entomol. Sci., Owashi, Tsukuba, Ibaraki 305-8634 Japan

SOURCE: Journal of Virology, (Feb., 1999) Vol. 73, No. 2, pp. 1219-1226.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English

AB AUG-unrelated translation initiation was found in an insect picorna-like virus, Plautia stali intestine virus (PSIV). The positive-strand RNA genome of the virus contains two nonoverlapping open reading frames (ORFs). The capsid protein gene is located in the 3'-proximal ORF and lacks an AUG initiation codon. We examined the translation mechanism and the initiation codon of the capsid

protein gene by using various dicistronic and monocistronic RNAs in **vitro**. The capsid protein gene was translated cap independently in the presence of the upstream cistron, indicating that the gene is translated by internal ribosome entry. Deletion analysis showed that the internal ribosome entry site (IRES) consisted of approximately 250 bases and that its 3' boundary extended slightly into the capsid-coding region. The initiation codon for the IRES-mediated translation was identified as the CUU codon, which is located just upstream of the 5' terminus of the capsid-coding region by site-directed mutagenesis. In **vitro** translation assays of monocistronic RNAs lacking the 5' part of the IRES showed that this CUU codon was not recognized by scanning ribosomes. This suggests that the PSIV IRES can effectively direct translation initiation without stable codon-anticodon pairing between the initiation codon and the initiator methionyl-**tRNA**.

L9 ANSWER 13 OF 44 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1999175320 MEDLINE
DOCUMENT NUMBER: 99175320 PubMed ID: 10074407
TITLE: Binding properties of the human immunodeficiency **virus** type 1 **nucleocapsid** **protein** p7 to a model RNA: elucidation of the structural determinants for function.
AUTHOR: Urbaneja M A; Kane B P; Johnson D G; Gorelick R J; Henderson L E; Casas-Finet J R
CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201, USA.. urbaneja@avpaxpl.ncifcrf.gov
CONTRACT NUMBER: N01-CO-56000 (NCI)
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Mar 19) 287 (1) 59-75.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990426
Last Updated on STN: 19990426
Entered Medline: 19990415

AB HIV-1 nucleocapsid protein (NCp7) is a double zinc-fingered protein that has been traditionally implicated in viral RNA recognition and packaging, in addition to its tight association with genomic RNA and **tRNA** primer within the virion nucleocapsid. The availability of large quantities of viral or recombinant wild-type NCp7 and mutant p7 has made possible the assignment of the different roles that structural motifs within the protein play during RNA binding. At low ionic strength binding to the homopolymeric fluorescent [illegible] poly(epsilonA), is electrostatically driven and four sodi displaced. Arg7 in the flanking N-terminal region, Lys20 in the first zinc finger and one positively charged resid (attributed to Lys41) in the second zinc finger are invol electrostatic contacts with RNA. The p7 zinc fingers do nc independently but concomitantly. The first zinc finger (bc isolated or in the context of the full-length protein) has prominent electrostatic interaction than the second one. T

zinc finger dominates the non-electrostatic stabilization of the binding to RNA due to stacking of its Trp residue with nucleic acid bases. Mutations in the highly conserved retroviral Zn-coordinating residues (CCHC) to steroid hormone receptor (CCCC) or transcription factor (CCHH) metal cluster types do not affect RNA binding. In spite of the limited impact in RNA binding affinity in **vitro** or RNA packaging in vivo that such mutations or structural alterations impart, they impair or abolish virus infectivity. It is likely that such an effect stems from the involvement of NCp7 in crucial steps of the virus life cycle other than RNA binding.

L9 ANSWER 14 OF 44 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998440607 MEDLINE
DOCUMENT NUMBER: 98440607 PubMed ID: 9765488
TITLE: Roles of the human immunodeficiency **virus** type 1 **nucleocapsid protein** in annealing and initiation versus elongation in reverse transcription of viral negative-strand strong-stop DNA.
AUTHOR: Rong L; Liang C; Hsu M; Kleiman L; Petitjean P; de Rocquigny H; Roques B P; Wainberg M A
CORPORATE SOURCE: McGill University AIDS Centre, Lady Davis Institute-Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2.
SOURCE: JOURNAL OF VIROLOGY, (1998 Nov) 72 (11) 9353-8. Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981105

AB To study the initiation of human immunodeficiency virus type 1 reverse transcription, we have used the **viral nucleocapsid protein** (NC7) to anneal tRNA3Lys primer onto viral genomic RNA and have then eliminated NC7 from this primer-template complex by digestion with proteinase K and phenol-chloroform extraction of residual protein. Our data show that saturating concentrations of NC7 resulted in the formation of an active **tRNA**-template complex that yielded enhanced production of full-length negative-strand strong-stop DNA [(-)ssDNA] and that this complex remained active even after the elimination of NC7. While both of the two Zn finger motifs found within NC7 were essential for efficient elongation, NC protein that contained a point mutation in the first Zn finger or that was devoid of both Zn fingers yielded primer-template complexes that could still be initiated in 1-base-extension assays. In contrast, the use of heat annealing to produce primer-template complexes resulted in proportions of full-length (-)ssDNA lower than those seen with NC protein, and the addition of NC protein to such preformed primer-template complexes was able to reverse this defect only to a marginal extent.

L9 ANSWER 15 OF 44 BIOSIS
ACCESSION NUMBER: 1998:428519 BIOSIS

DOCUMENT NUMBER: PREV199800428519
TITLE: The yeast Ty3 retrotransposon contains a 5'-3' bipartite primer-binding site and encodes nucleocapsid protein NCp9 functionally homologous to HIV-1 NCp7.
AUTHOR(S): Gabus, Caroline; Ficheux, Damien; Rau, Michael; Keith, Gerard; Sandmeyer, Suzanne; Darlix, Jean-Luc (1)
CORPORATE SOURCE: (1) LaboRetro, Unite Virologie Humaine, INSERM, Ecole Normale Superieure Lyon, 46 Allee d'Italie, 69364 Lyon France
SOURCE: EMBO (European Molecular Biology Organization) Journal, (Aug. 17, 1998) Vol. 17, No. 16, pp. 4873-4880.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Retroviruses, including HIV-1 and the distantly related yeast retroelement Ty3, all encode a nucleoprotein required for virion structure and replication. During an in **vitro** comparison of HIV-1 and Ty3 nucleoprotein function in RNA dimerization and cDNA synthesis, we discovered a bipartite primer-binding site (PBS) for Ty3 composed of sequences located at opposite ends of the genome. Ty3 cDNA synthesis requires the 3' PBS for primer tRNAiMet annealing to the genomic RNA, and the 5' PBS, in cis or in trans, as the reverse transcription start site. Ty3 RNA alone is unable to dimerize, but formation of dimeric tRNAiMet bound to the PBS was found to direct dimerization of Ty3 RNA-tRNiMet. Interestingly, HIV-1 nucleocapsid protein NCp7 and Tyr3 NCp9 were interchangeable using HIV-1 and Ty3 RNA template-primer systems. Our findings impact on the understanding of non-canonical reverse transcription as well as on the use of Ty3 systems to screen for anti-NCp7 drugs.

L9 ANSWER 16 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 1998:322473 SCISEARCH
THE GENUINE ARTICLE: ZJ231
TITLE: Endogenous reverse transcriptase assays reveal synergy between combinations of the M184V and other drug resistance-conferring mutations in interactions with nucleoside analog triphosphates
AUTHOR: Quan Y D; Gu Z X; Li X G; Liang C; Parniak M A; Wainberg M A (Reprint)
CORPORATE SOURCE: MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA (Reprint); MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA; MCGILL UNIV, DEPT MED, MONTREAL, PQ, CANADA; MCGILL UNIV, DEPT MICROBIOL & IMMUNOL, MONTREAL, PQ, CANADA
COUNTRY OF AUTHOR: CANADA
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (27 MAR 1998) Vol. 277, No. 2, pp. 237-247.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX.
ISSN: 0022-2836.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 56
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Resistance of HIV-1 reverse transcriptase (RT) to nucleoside analogs (e.g. AZT, ddC and 3TC) is conferred by various amino acid substitutions or combinations thereof on the RT molecule, The M184V mutation, that confers high and low-level resistance to 3TC and ddC, respectively, can restore sensitivity to AZT when introduced into RT against a background of AZT-resistance. The K65R mutation, that confers low level resistance to both 3TC and ddC, can also restore sensitivity to AZT. This information is of potential utility in choosing combinations of anti-viral drugs for clinical use. To explore this subject further, we have used an endogenous RT reaction to study mutated viruses containing M184V alone or M184V combined with each of the K65R, E89G or both the M41L and T215Y substitutions. Endogenous assays possess the advantage of utilizing genomic RNA as template in a reaction mixture that includes each of **tRNA**(Lys.3) and **viral nucleocapsid protein**, necessary for specific initiation of reverse transcription, as well as all other viral proteins that might impact on this process. We now show that viruses containing both M184V and K65R displayed synergistic resistance to 3TC triphosphate (3TCTP), while the same combination yielded the same level of resistance to ddC triphosphate (ddCTP) as that manifested by K65R alone. The combination of M184V and E89G displayed synergistic resistance against ddCTP but not 3TCTP, while viruses containing only E89G were highly resistant to 3TCTP and displayed low-level resistance to ddCTP. The results show that endogenous RT assays can reveal variable synergistic, antagonistic, or neutral effects in regard to drug sensitivity, depending on the presence of specific amino acid substitutions in RT itself. (C) 1998 Academic Press Limited.

L9 ANSWER 17 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 97:181293 SCISEARCH
THE GENUINE ARTICLE: WK242
TITLE: Ordered aggregation of ribonucleic acids by the human immunodeficiency **virus** type 1 **nucleocapsid protein**
AUTHOR: Stoylov S P (Reprint); Vuilleumier C; Stoylova E; DeRocquigny H; Roques B P; Gerard D; Mely Y
CORPORATE SOURCE: BULGARIAN ACAD SCI, INST PHYS CHEM, BG-1040 SOFIA, BULGARIA (Reprint); UNIV STRASBOURG 1, FAC PHARM, CNRS, URA 491, LAB BIOPHYS, F-67401 ILLKIRCH GRAFFENS, FRANCE; UFR SCI PHARMACEUT & BIOL, CNRS URA 498, INSERM U266, DEPT CHIM ORGAN, F-75270 PARIS, FRANCE
COUNTRY OF AUTHOR: BULGARIA; FRANCE
SOURCE: BIOPOLYMERS, (MAR 1997) Vol. 41, No. 3, pp. 301-312. Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0006-3525.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The nucleocapsid protein NCp7, which is the major genomic RNA binding protein of human immunodeficiency virus type 1, plays an important role in several keys steps of the viral life cycle. Many of the NCp7 activities, notably the nucleic acid annealing and the genomic RNA wrapping ones, are thought to be linked to a nonspecific